**University of Dundee**

**MASTER OF DENTAL SCIENCE**

# Maternal diabetes, obesity and oral clefts
# a pilot study

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MASTER OF DENTAL SCIENCE

# Maternal diabetes, obesity and oral clefts

*a pilot study*

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University of Dundee

# MATERNAL DIABETES, OBESITY AND ORAL CLEFTS:

## A Pilot Study


A thesis submitted for the degree of MDSc

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| | |
|---|---|
| BCL | Bilateral cleft lip |
| BCLP | Bilateral cleft lip and palate |
| BMI | Body mass index |
| CEMACH | Confidential Enquiry into Maternal and Child Health |
| CHI | Community Health Index |
| CL | Cleft lip |
| CLEFTSiS | Cleft Services in Scotland |
| CL(P) | Cleft lip with or without cleft palate, and cleft palate |
| CL/P | Cleft lip with or without cleft palate |
| CLP | Cleft lip and palate |
| CL | Cleft lip |
| CP | Cleft palate |
| CSAG | Clinical Standards Advisory Group |
| DCCT | Diabetes control and complications trial |
| DGI | Dietary glycaemic index |
| ECEMC | Spanish Collaborative Study of Congenital Malformations |
| ENT | Ear, Nose and Throat |
| EPR | Electronic patient record |
| ESF | European Science Foundation |
| EUROCAT | European Surveillance Systems of Congenital Anomalies |
| GDM | Gestational diabetes mellitus |
| GLUT3 | Glucose transporter 3 |
| HIC | Health Informatics Centre |
| HRQoL | Health Related Quality of Life |

| | |
|---|---|
| ICBDSR | International Clearinghouse for Birth Defects Surveillance and Research |
| IDCFA | International Database for Craniofacial Anomalies |
| IPDTOC | International Perinatal Database of Typical Oral Clefts |
| MCN | Managed clinical network |
| MetS | The Metabolic Syndrome |
| NBDPN | National Birth Defects Prevention Network |
| NGT | Normal glucose tolerance |
| NHS | National Health Service |
| NIH | National Institute for Health |
| NSCL(P) | Non-syndromic cleft lip with or without cleft palate, and cleft palate |
| NSD | National Services Division |
| OFC | Orofacial cleft |
| SCALP | Scottish Association for Cleft Lip and Palate |
| SCI-DC | Scottish Core Information – Diabetes Collaboration |
| SIMD | Scottish Index of Multiple Deprivation |
| SMR02 | Maternity Inpatient and Day Case |
| TOPs | Terminations of pregnancy |
| UCL | Unilateral cleft lip |
| UCLP | Unilateral cleft lip and palate |
| WHO | World Health Organisation |

## Appendices

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Thanks must also go to the team at HIC for gathering and anonymising the data. This project would not have been possible without their input, and particularly that of Mrs Alison Bell.

## DECLARATION

This thesis is the original work of the author

Unless stated, all references cited have been consulted by the author

This work has not been previously accepted for a higher degree

## ABSTRACT

**Background:** Orofacial clefts (OFC) are among the most common congenital anomalies worldwide and carry a great burden for those children affected. Recent studies have identified the possibility of positive links between maternal diabetes/abnormal glucose metabolism and maternal overweight/obesity with the occurrence of orofacial clefts in their offspring. Further investigation into these possible aetiological factors is warranted in order to inform preventative strategies against OFC.

**Aims:** The aim of this study is to investigate the association between oral cleft in children and abnormal glucose metabolism and obesity in their mothers in the Tayside population. This will provide unique pilot data to inform a future Scotland wide study.

**Design and Setting:** A case-control record linkage study undertaken at the University of Dundee during 2011–2013.

**Materials and Methods:** Information regarding 138 mothers of infants born with OFC in the Tayside area between 1990–2010 was identified and anonymised using the CLEFTSiS database. This was linked to further healthcare datasets held within NHS Tayside and compared with corresponding datasets regarding 564 matched control mothers. The possibility of a significant difference in the proportion of diabetic case mothers compared to control mothers was analysed using a chi-squared test. Possible differences between maternal glycosylated haemoglobin levels and weight were assessed for significance using an independent samples t-test.

**Results:** Completeness of datasets was found to be poor regarding maternal height/weight (and therefore BMI) and confounding factors including smoking status and alcohol intake at the time of delivery was also incomplete. No significant differences were found in the proportion of diabetic case and control mothers ($p$ =

0.6305). The recorded number of diabetic mothers was low in both groups (4/138 and 13/564 respectively). No significant differences were found in mean maternal glycosylated haemoglobin levels in diabetic mothers ($p$ = 0.368) or weight ($p$ = 0.418) between the cases and controls.

**Conclusions:** Datasets were found to be incomplete, and confounding factors were poorly documented. There is no identifiable link between maternal diabetes and OFC in babies born to these mothers within the Tayside population. No significant differences were identified regarding glycosylated haemoglobin levels in diabetic mothers or weight between case and control mothers. Larger and wide-reaching studies are required to overcome the limitations of a relatively small population sample. Datasets of maternal information must be complete and confounding factors fully documented to allow studies to be accurately performed.

## CHAPTER ONE: INTRODUCTION

Orofacial clefts (OFC) are among the most common birth defects worldwide, with an estimated prevalence of around 1 in 700 live births (1). For those individuals born with OFC, it will have a profound effect on their health and wellbeing and requires complex and lengthy treatment strategies.

Although much research has been carried out focussing on potential genetic and environmental causes of OFC, the aetiology of most cases remains unclear. The outcome of research studies must be to identity aetiological factors and thus inform preventative strategies for future generations (2).

Several recent studies have identified possible links between maternal diabetes and maternal obesity and the occurrence of OFC in offspring (3,4). These were conducted within a population in the USA.

It is therefore of interest to investigate the possibility of such a link within our own population. This will inform both the need for further wider-ranging studies and possible protective actions such as screening and pregnancy planning.

The larger and more wide-ranging the evidence base underpinning the aetiological factors leading to OFC, the greater the potential to develop such preventive strategies.

# CHAPTER TWO: BACKGROUND AND LITERATURE REVIEW

## 2.1 Orofacial clefting: Embryology, pathogenesis and aetiology

During embryonic development, any disruption in the fusion of tissues forming the lip and palate can result in clefting. The associated anomaly can manifest as either cleft lip with or without cleft palate or as isolated cleft palate.

In order to build a clear picture of the pathogenesis of orofacial clefting it is important to develop an understanding of the normal embryological development of the lip and palate.

### 2.1.1 Development of the face

Neural crest cells originating in the neuroectoderm migrate vertically and go on to form the facial skeleton and many further structures in this area. These cells are of vital importance in the development of the craniofacial structures and present a vulnerable target during the migration process.

Development of the human face commences during the fourth week in utero when migrating neural crest cells join with core mesoderm and the epithelial cover to form the facial primordial (5). The primitive mouth is called the stomodeum.

The stomodeum is surrounded by the five facial prominences, consisting mainly of neural crest cell derived mesenchyme and formed from the first pharyngeal arch. This occurs by the end of the fourth week. These swellings are comprised of the frontonasal prominences (upper border of stomodeum), paired maxillary

prominences (lateral to stomodeum) and paired mandibular prominences (caudal to stomodeum). By five weeks in utero the nasal placodes are formed to either side of the frontonasal prominence by local thickening of the surface epithelium (figure 1) (6).

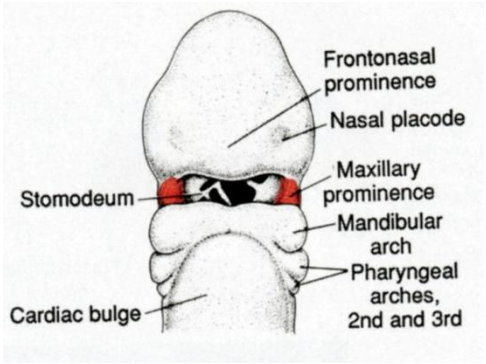



**Figure 1: Frontal view of an embryo at 4.5 weeks, adapted from (Sadler, 2006)**

The primitive nasal cavities appear during the fifth week as the nasal placodes invaginate into underlying mesenchyme, thus the nasal pits are formed. The ridges of tissue surrounding each pit form from proliferating mesenchyme of the frontonasal processes and are known as the nasal prominences. The lateral nasal prominences situated on the outer edge of each nasal pit and medial nasal prominences on the inner edge.

As the nasal pits continue to proliferate and submerge, they are held separate to the stomodeum by the oronasal membrane until its regression at the end of the fifth week (figure 2).

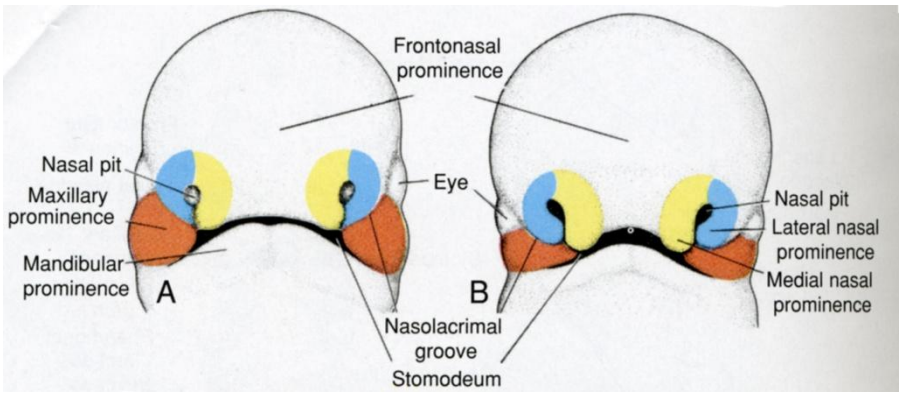



**Figure 2: Frontal view of the developing embryo during the A. fifth week B. sixth week in utero, adapted from (Sadler, 2006)**

In weeks six and seven the lower lip and mandible form following the merging of the mandibular prominences across the midline. The maxillary prominences continue to grow towards the medial nasal processes causing their compression towards the midline. Loss of the cleft between the medial nasal processes and the maxillary prominence occurs as they fuse together (figure 3).

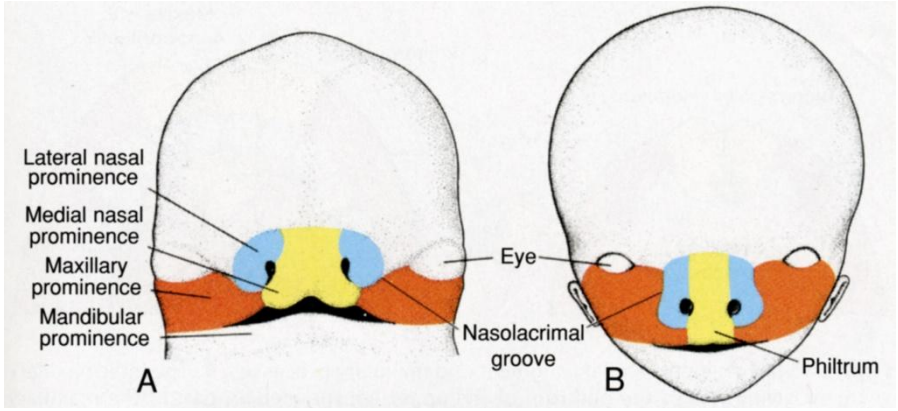



**Figure 3: Frontal view of 7-week embryo. Maxillary prominences have fused with the medial nasal prominences. B. 10-week embryo, adapted from (Sadler, 2006)**

Some debate exists regarding the formation of the upper lip and primary palate. Because the upper lip is innervated by the trigeminal nerve, some believe it must form entirely from the maxillary processes which ‘overgrow’ the medial nasal processes. The nerve supply would come from the ophthalmic nerve if formation had arisen from the frontonasal process (7). Others believe that the maxillary processes do not overgrow the medial nasal processes; rather a part of the frontonasal process persists to form the philtrum of upper lip. However this does not explain the innervations of the upper lip by the trigeminal nerve (8).

The fusion of the maxillary processes with the medial nasal processes forms a structure called the ‘intermaxillary segment’. Any teratogenic agent or disruption of growth may influence this vulnerable period of cell division, resulting in failure of fusion and the formation of a cleft of the lip and/or alveolus. The intermaxillary segment is composed of the labial component, the maxillary component and the palatal component, which goes on to form the primary palate.

### 2.1.2 Development of the secondary palate

The secondary palate begins to form during the sixth week in utero. The lateral palatine shelves form as outgrowths from the internal aspect of the maxillary processes. The nasal septum separates the nasal cavities and a secondary nasal septum continues to grow downwards separating the oronasal cavity.

The lateral palatine shelves grow downwards vertically, lateral to the tongue; which is the dominant structure in the oronasal cavity (figure 4).

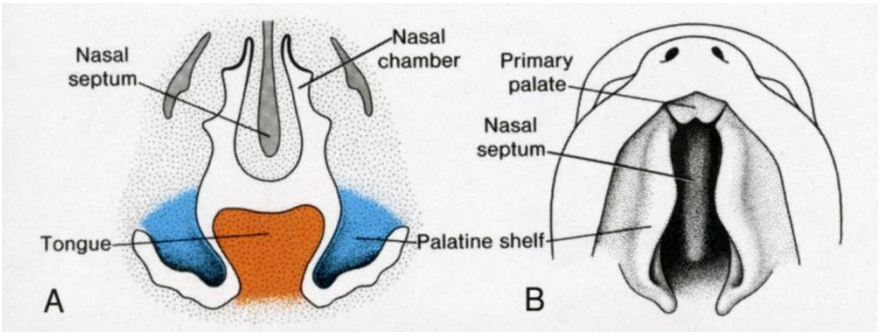



**Figure 4: 6-week-old embryo in section (A). The palatine shelves are in the vertical position on each side of the tongue (B). This shows clefting between the primary triangular palate and the palatine shelves, which at this stage are still vertical, (adapted from Sadler 2006)**

During the seventh week, the palatine shelves rise into a horizontal position above the tongue. The tongue musculature gains the ability to contract, becomes depressed and moves downwards. The lateral palatine shelves become turgid, elevate and then fuse to form the secondary palate. Formation of the secondary palate can only come about if a series of events occur without complication. The head elevates upwards away from the developing chest, the volume of the stomodeum increases and as previously mentioned the tongue drops in position (figure 5) (8).

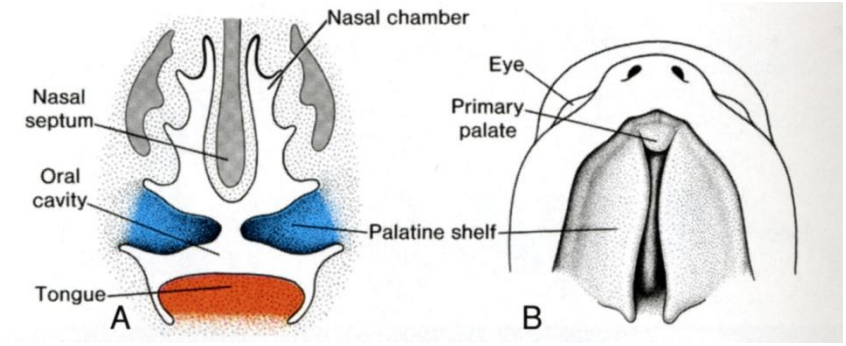



**Figure 5: Elevation of the lateral palatal shelves in a 7.5-week embryo, adapted from (Sadler, 2004)**

The lateral palatine shelves fuse not only with each other, but also with the primary palate, with the incisive foramen demarcating the point of fusion. This union acts to separate the oral and nasal cavities and enables the simultaneous functions of respiration and mastication. The nasal septum continues downwards growth to fuse with the newly formed palate (figure 6).

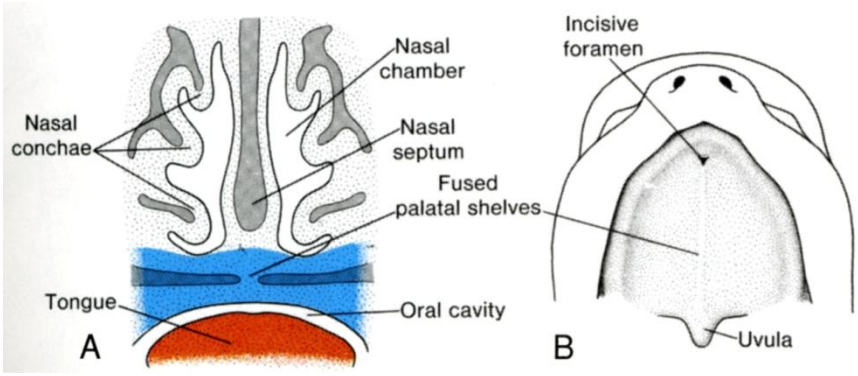



**Figure 6: Fusion of the palatine shelves and the nasal septum in a 10-week embryo, adapted from (Sadler, 2006)**

During approximation of the palatal shelves, the medial edge epithelium forms a midline epithelial seam which rapidly degenerates, establishing continuous mesenchyme across the midline. This occurs through a process of cell death, migration and transformation (7). Fusion of the secondary palate is normally complete by the twelfth week in utero (9), however as horizontal positioning of the palatal shelves occurs later in the female embryo, there is an extended period during which the open palatal shelves may be susceptible to teratogenic influences and therefore a possible correlation with the greater incidence of isolated cleft palate in females (10).

During the period of palatal shelf elevation there is almost no increase in head width while growth in head height continues (11) and therefore the expanding palatal shelves are able to grow above the tongue and fuse. However, if palatal shelf

elevation is delayed until the pattern of facial growth takes a horizontal direction there may be a failure of palatal shelf contact, and subsequently a failure of fusion.

Much existing knowledge of the development of the secondary palate comes from studies of mice due to the similar morphological chronology to humans (12). Regulation of palatal shelf initiation and growth is believed to involve complex signalling cascades entailing transcription factors, growth factors and their receptors, including Osr2, Lhx8, Msx1, Fgf10, Fgfr2b, Tgfb2 and Tgfbr2 (13). Palatal growth is regulated by signalling between the palatal epithelium and mesenchyme, involving fibroblast growth factor 10 (FGF10) and the receptor FGFR2b found on the palatal epithelium. Failure of this communication due to loss of function of signalling factors leads to reduced mesenchymal proliferation and a rise in apoptosis. This subsequently causes a shortening of the palatal shelves, leaving them unable to fuse.

Sonic hedgehog (SHH) expression in the palatal shelves is vital and any loss of SHH function can lead to clefting of the palate (14). Maintenance of SHH expression is related to activation of FGFR2b by FGF10, further highlighting their importance in palatal development. Growth of the palatal shelves is stimulated by SHH (15). Furthermore, a loss of function mutation in the gene MSX1 has been identified in patients affected by cleft lip and palate (16).

### 2.1.3 Elevation of the palatal shelves

There have been many theories proposed to explain the mechanism of palatal shelf elevation and it is still not wholly understood.

The most plausible explanation is that of an intrinsic force generated within the palatal shelves themselves. When this force reaches a threshold exceeding that of its resistance (i.e. from the tongue), elevation of the palatal shelves can occur (7). Shelf elevating force appears to be due to a regional accumulation of glucosaminoglycans, mainly hyaluronic acid. This highly electrostatically charged molecule can bind up to ten times its own weight of water. Epidermal growth factor stimulates the synthesis of hyaluronic acid by palatal mesenchymal cells, which swell to produce an elevating force. Differential proliferation and alignment of the mesenchymal cells may add further elevating force and act to influence the direction of elevation (7)(17).

The control of palatal shelf adhesion is vital in ensuring the palatal shelves rise and do not adhere to the wrong structures. When the shelves are vertical and in close proximity to other structures they are ‘adhesion incompetent’, however once positioned horizontally above the tongue they are able to adhere to one another and thereby prevent clefting. The membrane bound signalling molecule JAG2 and interferon regulating factor 6 (IRF6) have been found to be highly influential in this process (12,18,19).

### 2.1.4 Fusion of the palatal shelves

Once elevated, the lateral palatal shelves contact each other and also the primary palate and nasal septum. The epithelial cells of the medial edges adhere due to a sticky glycoprotein coat. These become the central cells of the epithelial seam (7).

Fusion of the palatal shelves appears to be stimulated by cell-adhesion molecules, desmosomal components and growth factors such as transforming growth factor α (TGFα) and epidermal growth factor receptor (EGFR) plus those in the transforming growth factor β family such as TGFβ3 (12). TGFβ3 may play a vital role in palatal fusion as it is expressed in the medial edge epithelium prior to and during fusion (20).

Once the medial epithelial seam has formed following rapid assembly of desmosomal components, there must then be degeneration of the seam to provide continuity of mesenchyme across the midline. It would appear that programmed cell death (apoptosis) plays a key role in seam degeneration and dead, dying cells and those primed for cell destruction have been identified in the disintegrating medial epithelial seam (21). There has been much debate regarding the possibility of epithelial-mesenchymal transformation, however evidence supports a theory of epithelial cell migration not transformation (22).

Following these events there is differentiation of the tissue on the oral and nasal aspects of the palate. Within the fused palatal shelves intramembranous ossification commences, developing the future hard palate.

### 2.1.5 Formation of cleft palate

Any disturbances during the phase of palate development can lead to the formation of cleft palate:

- Defects in palatal shelf growth
- Delayed or failed shelf elevation
- Defective shelf fusion
- Failure of medial edge cell death
- Post fusion rupture
- Failure of mesenchymal consolidation and differentiation (9)

If there is a disruption in the formation of both the primary and secondary palate, cleft palate may be associated with clefting of the lip and alveolus. Cleft lip and palate can also each arise in isolation and have differing embryological origin (23).

Clearly, the time period during which the lip and palate is developing involves a complex series of events, and any influence from disruptive factors may lead to significant anomalies such as cleft lip and palate.

### 2.1.6 Environmental aetiology of OFC

The findings from epidemiological and experimental studies of OFC implicate numerous environmental factors in the development of clefts of the lip and/or palate:

- Maternal exposure to tobacco smoke/ maternal smoking
- Maternal alcohol intake
- Nutritional deficiency
- Drug intake including anticonvulsants and steroids
- Maternal systemic illness, elevated maternal blood glucose and obesity

#### Smoking

Maternal smoking during the first trimester of pregnancy has been linked with increased risk of a baby being born with CL/P and isolated CP (24,25). A meta-analysis of 24 studies found 'consistent, moderate and statistically significant associations between both CL/P and CP and maternal smoking' (26). Mothers who smoked during pregnancy had a 1.3-fold risk of having a baby with CL/P and a 1.2-fold risk of CP alone. A case-control study in the UK by the same author found a 'small but statistically significant' link between maternal smoking during the first trimester of pregnancy and risk of OFC (27). Maternal exposure to passive smoking may not be well reported, and consequently not assessed in many studies, therefore the link between exposure to smoke and OFC may be underestimated (12).

**Alcohol**

Currently, any link between maternal alcohol intake and non-syndromic oral clefting is unclear. Its assessment in previous studies has proven difficult due to several factors. It may be problematic to quantify 'alcohol consumption' as reported by individuals. The confounding effects of other environmental factors such as smoking, nutrition and drug use must be considered, and alcohol may also interact with and modify the effects of these other environmental factors. Positive associations between maternal alcohol consumption and OFC have been found in some studies (28-30), but not in others such as Romitti et al in 2007 (31), however the results of the former may have been influenced by confounding factors.

**Nutrition**

A possible link between maternal nutrition and OFC has been identified through observational studies, however accurate assessment of nutritional status can prove problematic, particularly in poorer populations where the highest rates of OFC are seen (12).

A meta-analysis by Johnson and Little (2008) found 25% reduction in births affected by OFC when the mothers had taken multivitamin supplements (32). However these findings may be influenced by confounding factors such as a heightened awareness of health and positive healthy behaviours in these mothers. Small sample sizes in previous studies may also skew results.

Whether or not dietary intake of folic acid offers protection from OFC remains uncertain, despite the results of case-control studies of maternal dietary folate uptake, multivitamin supplements containing folic acid and red cell plasma folate levels (12).

**Drug therapy**

Several studies have identified a link between anticonvulsant drugs taken to control maternal epilepsy, including diazepam, phenytion and phenobarbital and OFC (33-35). The use of corticosteroids during pregnancy has also been associated with OFC (36), as have analgesic and antipyretic medications (37).

**Maternal diabetes and obesity**

The possible associations between OFC and elevated maternal blood glucose, and maternal diabetes and obesity form the focus of this study and will therefore be discussed at length further in this review.

**Other risk factors**

Other factors found to have a positive association with OFC include environmental pollution, e.g. domestic due to poor ventilation during cooking and heating, consumption of pickled vegetables more than six times per week and a history of fever and cold (37). Viral infection may be relevant in development of OFC as interferon regulatory transcription factors are activated following viral infection, and IRF6 has been seen to be associated with clefting (38).

**Summary**

Numerous studies have implicated a wide range of possible environmental factors in the aetiology of cleft lip and palate. It has been difficult to separate these factors and ascertain their individual effects. Additionally they may act to modify to other factors.

Future studies may aim to separate these factors where possible to analyse theit individual effects.

## 2.2 Genetics and OFC

Whether identified as CL/P or isolated CP, orofacial clefting can also be classified as non-syndromic OFC, clefts associated with chromosomal and monogenic syndromes, and clefts found in infants with multiple congenital anomalies (MCA) (39-41). Around 30% of CL/P and 50% of CP patients have some other congenital anomaly or an associated syndrome (42). If a cleft occurs in an individual who has other consistently related features, it can be termed as syndromic, with CL/P found in over 200 genetic syndromes and CP in over 400 (43).

Genetic factors are thought to play an important role in the aetiology of OFC, with 20% of patients showing a positive family history (44). Recent advances in quantitative and molecular analysis have made association and linkage studies of the aetiology of OFC possible (45).

Several previous studies have added to our knowledge of the genetic components of OFC development. The work of Fogh-Andersen first provided population-based evidence of a strong genetic component in OFC (46). However the results of historical studies may have been affected by the aggregation of samples from differing geographical, racial or ethnic origins, or by small family sample groups (44).

It can be agreed that orofacial clefting has a multifactorial aetiology with genetic and environmental influencing factors. Where specific genetic disorders are excluded,

there remains a greater recurrence risk to siblings of those affected than can be predicted by aggregation of familial risks (47). There are increased concordance rates of OFC for monozygotic twins over dizygotic twins (48), and epidemiological studies consistently reveal increased rates of clefting and predominance of left sided clefting in males, further indicating a key role for genetic inheritance in OFC (49). Clefts of the primary palate that involve the lip and/or palate may also feature a different underlying mechanism to those of the secondary palate (50).

Studies utilising the technique of segregation analysis have thus far yielded a relatively small number of genes to be implicated with OFC, with a study by Fitzpatrick and Farrell in the West of Scotland identifying three or four major loci (51) and another study in England revealing two to fourteen loci (52).

Research focussing on the causes of OFC must take into account the complex and multifactorial aetiological picture, involving the identification of individual genes and the effect of gene-environment interactions. Several genes that play an important and interactive role in up to 20% of all clefts have recently been identified (42), however it is apparent that further causative genes remain unidentified.

Existing studies have used association and linkage analysis to investigate the influence of candidate genes in the aetiology of OFC; however a lack of consistency in results may be attributed to small sample groups or genetic heterogeneity (12,42). The difficulties in studying the genetics and associated environmental interactions have been further reflected in inconsistent results from population-based association studies. IRF6 had been the only gene to be consistently linked with non-syndromic CL(P) until 2009 (2). Genome-wide association studies (GWAS) may now provide an exciting opportunity to expand our knowledge of the genetic causes of OFC (53-56)

The interaction of aforementioned environmental risk factors with certain genes, and the variation with these genes, has an impact on the risk for OFC. There is a possibility that a developing foetus has a low genetic risk of developing OFC, however this risk is increased due to the environmental factors to which the mother is exposed and to her metabolism and inherent ability to detoxify these exposures (42).

Following the results of studies of monozygotic twins, finding levels of concordance for OFC below 100%, the hypothesis that OFC is not caused by genetic factors alone is further supported (50). Genetic and environmental factors may act in isolation or in synergy, therefore the study of gene-environment interactions (GEIs) can greatly add to our understanding of the aetiopathogenesis of OFC. Finding from such studies can provide guidance for global public health strategies, as it is much less contentious and theoretically possible to influence an individual's environment rather than their genetic profile. Strategies to alter environmental risk factors may be especially significant where those with a susceptible genetic profile can be identified. Although several potentially important GEIs have been investigated, currently the results are uncertain and have not been consistently replicated (12).

## 2.3 Epidemiology of OFC

Orofacial clefts are among the most common birth defects seen on a worldwide level, with incidence varying widely between different populations. Although the variation in geographical distribution of OFC due to differing birth prevalence has been widely recognised, the larger picture is not complete due to a lack of consistency and completeness in the recording of births and deficiencies in birth defect detection frameworks. There are also issues with source population of births

recorded, method of ascertaining data, time frame, sampling fluctuation and differences in inclusion and exclusion criteria. Some areas of the world are represented by very little information regarding the frequency of OFC, including parts of Eastern Europe, Africa and Asia (57).

The available data from a comprehensive overview of OFC epidemiology (58) indicates that OFC occurs in around 1 in 700 live births, but with significant ethnic and geographic variation (1). The WHO International Collaborative Research on Craniofacial Anomalies project aims to address the deficiencies in birth defect surveillance, particularly in the developing world (12).

### 2.3.1 Global studies

Worldwide, rates of detection of isolated CP may differ to those of CL/P due to the readily identifiable external phenotypic appearance of cleft lip. There is considerable variation in the incidence of CLP between different populations globally. Latin America, China and Japan have high rates of CL/P, while rates are low in Southern Europe, South Africa and Israel. Rates of isolated CP are elevated in Northern Europe, particularly Finland, and Canada while low rates of incidence are found in Latin America and South Africa (12).

The International Perinatal Database of Typical Orofacial Clefts (IPDTOC) was established in 2003 and collates data on CL/P and isolated CP from the European Surveillance Systems of Congenital Anomalies (EUROCAT) in Europe, the National Birth Defects Prevention Network (NBDPN) in the USA and the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR) worldwide. The 2011 IPDTOC working group publication provided data from 54 registries in 30 countries, covering at least one complete year from 2000-2005. This comprised

over 7.5 million births. 7704 cases of CL/P were identified, including 237 still births, 301 terminations of pregnancy (TOPs) and 25 with unknown pregnancy outcome. This gave an ‘overall’ prevalence of 9.92 per 10,000 for CL/P. The prevalence of CL was 3.28 per 10,000 and that of CLP was 6.64 per 10,000.

While 12 registries had a prevalence of CL/P statistically higher than the overall estimate of 9.92 per 10,000, 13 registries had a lower rate. The areas of highest prevalence were in Germany, Japan and Denmark, while the lowest rates were in Italy, South Africa and the USA.

Data may have been skewed due to registries that did not record TOPs. The areas reporting the highest rates of TOPs were the British Isles (16.7%), South-Mediterranean Europe (15.7%) and Western Europe (11.7%). Most TOPs were registered in multiple malformed cases, while they were rare in isolated cases of CL/P (59).

There is considerable variation in the prevalence of OFC in Europe. Prevalence of CL/P ranges from 3.4-22.9 per 10,000 births, while that of isolated CP showing greater variation with a range of 1.3-25.3 per 10,000 (58). These differences between European countries are believed to be true differences, and therefore not due to variable ascertainment rates, due to the consistent procedures involved with data collection for the EUROCAT registry (1). The European birth prevalence of cleft lip and palate is shown in figure 7.

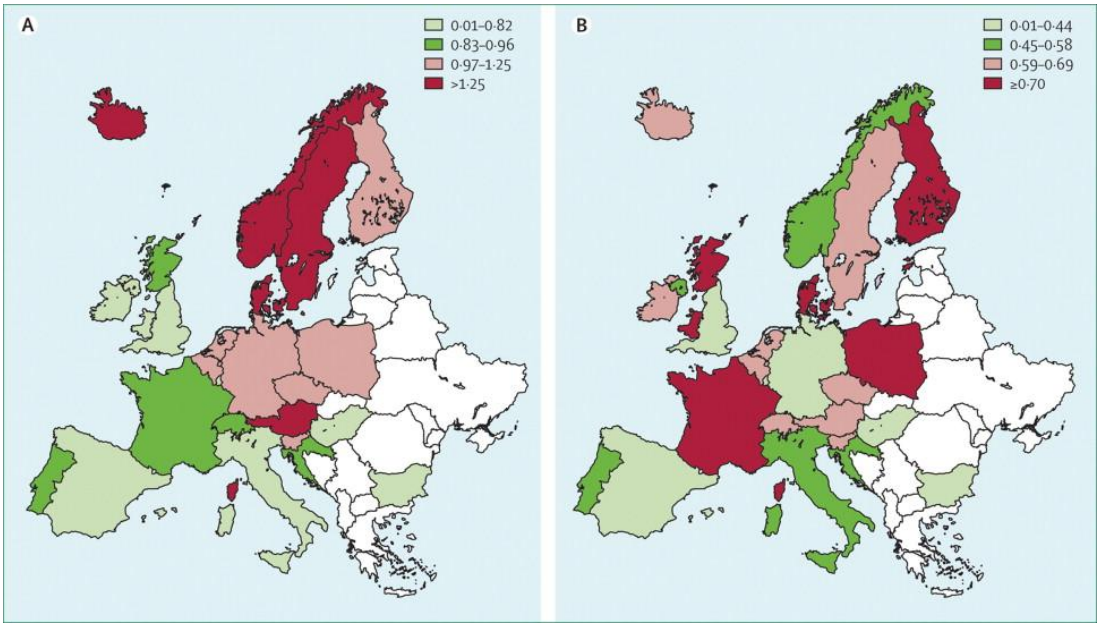



**Figure 7: European birth prevalence per 1000 live births of non-syndromic cleft lip and palate. (A) CL/P. (B) Isolated CP. (from http://www.eurocran.org/)**

Studies of immigrant groups to the USA from China and Japan suggest that the rates of CLP in these groups are more closely related to their area of origin compared to the region to which they have migrated (60,61). This indicates a genetic component which may interact with environmental factors in the aetiology of OFC.

Prevalence of CL(P) in Europe has been seen to positively correlate with South to North geographic location (59). There is speculation that this may be influenced by differing facial shape (62) and to levels of exposure to the sun with regards to vitamin D production.

### 2.3.2 Sex distribution

The differences in prevalence of OFC between males and females are well documented. The male sex has a tendency to CL/P, with a ratio of 2:1 reported in

white ethnic groups (49), while a study of a Scottish population found a 1.8:1 ratio (63). Females have a predilection towards isolated CP (39), this may be at least in part to the later elevation of the palatal shelves in the female embryo which provides a greater timeframe in which disruptive influences may act (9).

### 2.3.3 Cleft types and associated malformations

CL/P and isolated CP are often found in association with other congenital anomalies. This appears to be the case more frequently when considering isolated cleft palate (49). It may be the case that the presence of more obvious defects affecting other areas of the body may lead to detection of a cleft palate which may have otherwise remained undiagnosed.

A study of nearly 4000 cases of isolated CP in Europe found that 55% were isolated, 18% were associated with other anomalies and 27% were a feature of a recognised syndrome (64). This study also found that in over 5000 cases of CL/P 71% were isolated, while 29% were associated with other anomalies.

It has been suggested that incidence of OFC should be reported separately for live births, still births and terminations of pregnancy, and also divided from clefts with associated anomalies. This is because TOPs with CL(P) have more severe associated malformations, and the chance of OFC having developed in cases of still birth and TOPs is around three times that seen in live births. Cleft cases found with associated anomalies feature a different epidemiological distribution to non-syndromic cases of OFC (1).

It has been noted that where the prevalence of CL(P) is highest globally, the ratio of CLP to CL is also highest. This was first described by Mossey and Little (2002) and

further reinforced by the IPDTOC report. This supports a multifactorial model, which predicts that as the overall level of CL(P) in a population increases there is a greater genetic liability within that gene pool, leading to greater rates of CLP as opposed to CL (1,59).

Some evidence suggests an increased rate of additional malformations where bilateral clefts exist in comparison to unilateral clefts (65). The most commonly identified anomalies occurring in combination with OFC are congenital heart disorders and limb/vertebral column defects, although the genetic input remains unknown (1).

### 2.3.4 Future work

In order to develop strategies for future primary prevention of OFC, aetiological research must be supported by investigation into associations and trends within and between populations by epidemiological study. As it is already widely recognised that non-syndromic OFC has a complex polygenic multifactorial basis with ethnic, racial and geographical variation, investigation of these factors at a population level is vital in building the bigger picture of OFC aetiology and supporting work towards ascertaining methods for possible future prevention.

## 2.4 The impact of OFC

When a baby is born with CL(P), there will follow many questions, issues and consequences for the individual, their family and those within their social sphere. Many of these issues can be life-long, involve difficulties, expense and stigmatisation, particularly the social isolation found in some parts of the world.

Parents of a child born with OFC often have feelings of guilt, isolation, depression and disbelief. The immediate effect on parent/child bonding may have important consequences.

### 2.4.1 Feeding

The communication between the oral and nasal cavities in babies born with cleft palate poses a problem for feeding. Jones (1988) reported significant feeding problems and lower mean weight gain in a quarter of babies with non-syndromic OFC studied (66). Another study found that one third of newborns had problems with feeding; however this effect did diminish over time (67). Both studies noted that those individuals with the greatest experience of feeding difficulty had CP or CLP, while those CL had the least difficulty.

Britton et al (2011) found that in the Scottish population, 54% of children with OFC were breast-fed at birth, in comparison to 70% of non-cleft babies. Those with CL were more likely to be breast-fed than those with CP (68).

### 2.4.2 Hearing

Deficient middle ear drainage due to Eustachian tube dysfunction leads to hearing problems in individuals born with OFC affecting the posterior palate. A lack of muscular activity in the tensor veli palatine muscles of the soft palate causes the defective drainage, which may lead to recurrent infections of the middle ear and eventual rupture of the ear drum. Ventilation tubes (grommets) are routinely placed to prevent these problems arising. In a study of patients with *unrepaired* palatal clefts, up to 60% suffered from hearing impairment (69).

### 2.4.3 Speech

Defective speech may occur due to velopharyngeal insufficiency and can also be affected by poor hearing. Velopharyngeal insufficiency arises when the soft palate is unable to contact the posterior pharynx and close off the nasal airway. Although surgery improves speech in the majority of individuals with OFC, around 25% fail to develop adequate speech (70). Health Related Quality of Life (HRQoL) is significantly impaired in those with severe speech problems, and therefore this consequence of OFC may pose major issues for the individual and those around them (71).

### 2.4.4 Dental anomalies associated with OFC

The dental anomalies found in association with CLP include delayed dental development and eruption, impactions, hypodontia, enamel defects and anomalies of tooth size and shape.

Tortura et al (2008) found 48% of patients with UCLP to have a missing lateral incisor on the cleft side, with only 6% on the non-cleft side. They also reported an increased incidence of supernumerary or supplemental lateral incisors in these individuals (72). A twenty-fold increase in the risk of maxillary canine impaction has also been described (73). Orthodontic input is required from the early stages of an affected child's life and will continue for many years.

Scarring of the maxillary tissues following palate repair may cause maxillary arch restriction and present clinically as an anterior and/or posterior crossbite (74) . A combined orthodontic/orthognathic approach may be undertaken in order to correct the skeletal anomaly and associated malocclusion. This of course adds considerably to the burden of care.

### 2.4.5 Summary

There are significant challenges and costs which affect the life of a child born with OFC and also those around them and who care for them. Although much work has focussed on the management and assistance of those affected by OFC, the ultimate objective must be primary prevention.

## 2.5 Health inequalities in orofacial cleft care

There is considerable variation in rates of mortality and levels of access to care between, and also within, different countries worldwide. There is also variation in the quality of care and many children may be left affected by residual deformity and disability for life. In some cases clefts may remain unrepaired into adulthood. This is a significant problem in India, where 76% of patients with un-operated cleft palate suffer from mild to moderate conductive deafness (75) and very few achieve normal speech (76). Because of limited access to medical and surgical care in India, there is a perception that OFC is a life-threatening deformity. There may be reduced awareness that surgical repair is possible. Subsequently, many infants with OFC succumb to infection and malnutrition.

Surveillance systems for birth defects are generally deficient in India, with stark differences between rural and hospital based studies of prevalence. Rates of OFC prevalence may be found to be low in comparison to other populations, but this may be attributed to high rates of infant mortality. It is therefore important to achieve a precise estimate of the prevalence and distribution of OFC in the Indian population in order to target public health measures and reduce morbidity and mortality.

One indication of access to treatment is the age range of individuals with CLP receiving primary cleft surgery. It can be seen that in richer areas with better access to early surgery and treatment there is a much higher percentage of successful outcome (see figure 8). This highlights health inequalities between racial, ethnic and geographical groups.

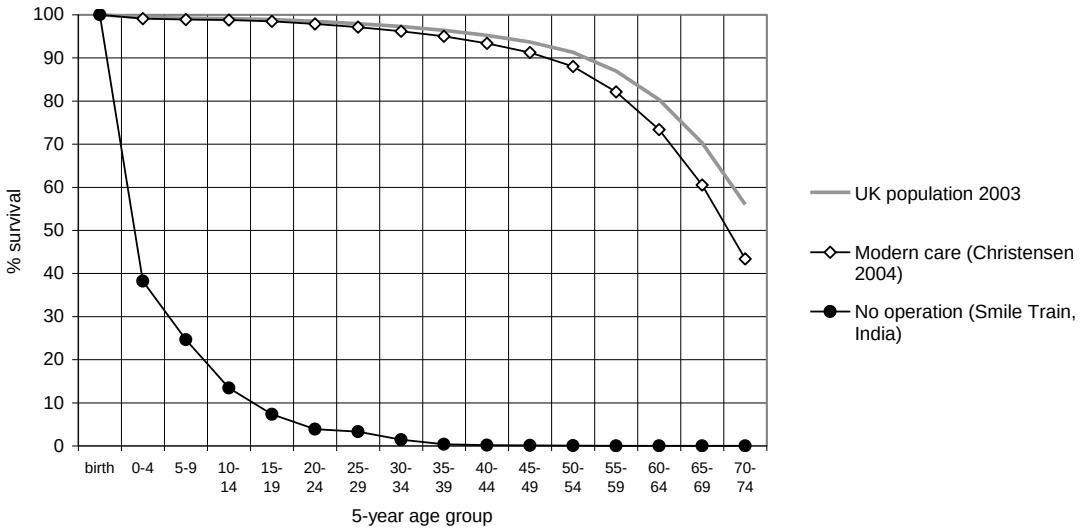



**Figure 8: The upper curve shows long-term survival of patients with orofacial clefts repaired in infancy: there is modestly increased all-cause mortality at all ages (Christensen et al 2004). The lower curve shows estimated survival with unoperated oro-facial clefts in India, based on Smile Train data. Adapted from** (1)

Of course health inequalities also exist in our own population and it is known that factors such as social class and negative health behaviours are related to other chronic health problems such as diabetes and obesity. This is known as the 'common risk factor' approach and was outlined in the Marmot Health Inequalities Review (2008) (http://www.ucl.ac.uk/gheg/marmotreview/).

Irregularities of care and service organisation are global issues, and therefore the WHO has stressed the requirement for strategies to improve clinical care through international collaboration between cooperatives. Outcome measures such as psychological and quality of life statuses require urgent attention.

## 2.6 Cleft registers and UK studies

Clinical cleft registries provide vital sources of data which can be used to inform important research, including epidemiological and genetic studies. The importance of registries in providing the framework for a collaborative network that can inform descriptive epidemiology, raise public awareness of health issues and support education, training and research has been highlighted by the WHO (58).

An advantage of using databases for epidemiological research is that populations can be studied in a relatively short period of time; however sample size should be adequate, and where the birth rate of the characteristic being studied is less than 2000 per year, at least ten years of data should be analysed (58).

Registries are either hospital based or population based, with the disadvantage of hospital based registries having potentially skewed data due to movement within regions at attend hospitals elsewhere and limitations of hospital access to those lacking in resources and living in deprived areas.

The importance of consistent protocols within the structure of population registries has been stressed by the WHO. Core areas to be input in cleft registries include differentiation between syndromic and non-syndromic OFC. The European Science Foundation (ESF) Common Core Protocols Project – Minimum Data Sets has proposed the recording of demographic information such as lifestyle factors and any existing pre-natal diagnoses, and the usage of a coding system for OFC that is internationally recognised and reproducible.

The International Database for Craniofacial Anomalies (IDCFA) was set up under the guidance of the WHO in order to collate data from the existing 62 registries that

are amenable to use for research and collects information on around 2 million births per year (77).

One of the greatest challenges to be overcome when aiming to build a global database of craniofacial anomalies is the collection of data from poor countries where standardisation of healthcare and data collection may be deficient. There may also be difficulties in the coding of anomalies, method of data collection and omissions due to terminations of pregnancy.

Within the UK, various studies based on databases and registries have reported on rates of birth prevalence of CL(P) (Table 3).

**Table 1: Birth prevalence of CL(P) in the UK, adapted from** (78)

| Authors | Source | No. of clefts | Region | Time span | No. of births | Birth prevalence (per 1000 live births) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | CL(P) | CP | All clefts |
| McMahon and Mckeown, 1953 | Clinical records | 285 | Birmingham | 1940-50 | 218,693 | | | 1.3 |
| Knox and Braithwaite,1962 | Hospital records | 574 | Northumberland | 1949-58 | 404,124 | 0.95 | 0.47 | 1.42 |
| Leck 1969 | Registry | 353 | Liverpool | 1950-59 | 186,046 | | | 1.9 |
| Owens et al, 1985 | Registry | 456 | Liverpool | 1960-82 | 325,727 | | | 1.4 |
| Leck and Lancashire, 1995 | Registry | 718 | Birmingham | 1960-84 | 432,778 | 1.04 | 0.62 | 1.66 |
| Womersley and Stone, 1987 | Registry | 247 | Glasgow | 1974-85 | 158,333 | 0.75 | 0.81 | 1.56 |
| Coupland and Coupland, 1988 | Hospital activity analysis | 930 | Trent | 1973-82 | 617,940 | | | 1.51 |
| Fitzpatrick et al, 1994 | Regional database | 286 | W. Scotland | 1980-85 | 187,321 | 0.74 | 0.79 | 1.53 |
| Gregg et al, 1994 | Regional database | 398 | N. Ireland | 1980-90 | 310,838 | 0.6 | 0.68 | 1.28 |
| Bellis and Wohlgemuth.B, 1999 | Regional database | 503 | S.E. Scotland and Highlands | 1971-90 | 356,922 | 0.77 | 0.63 | 1.4 |
| Cousley and Roberts-Harry, 2000 | Regional database | 132 | Yorkshire | 1994-95 | 82,265 | 0.88 | 0.73 | 1.6 |

### 2.6.1 CLEFTSiS

The National Managed Clinical Network for Cleft Service in Scotland (CLEFTSiS) has been collating data on OFC in Scotland since 1989. Prior to the year 2000, demographic data on OFC in Scotland was held by the Scottish Association for Cleft Lip and Palate (SCALP); however the outcomes of the Clinical Standards Advisory Group (CSAG) report of cleft services in the UK lead to the National Services Division (NSD) commissioning the CLEFTSiS network to succeed into this role.


**Figure 9: CLEFTSiS logo**

The CLEFTSiS mission statement reads: "Every patient with a cleft lip, cleft palate or cleft lip and palate is offered specialist cleft care from diagnosis to adulthood. We work with the family to offer the right care, in the right place at the right time to produce the best possible outcome for the patient" (http://www.cleftsis.scot.nhs.uk/).

When a child is born with CL(P) in Scotland, the CLEFTSiS team is notified within 24 hours. A visiting clinician will then complete a form (Appendix I) that records details regarding the patient and their parents. This is subsequently entered into the EPR with a completed consent form (Appendix II), which will soon be replaced with an information leaflet.

The aim of CLEFTSiS is to ensure interdisciplinary care for those affected by OFC, including access to care that meets agreed clinical standards, the planning of future care, the provision of clinical governance frameworks and to maintain the electronic patient record (EPR). The CLEFTSiS EPR records information regarding all live births with OFC in Scotland and its purpose is to maintain a record of all clinical activities and interactions for each patient in order to allow joined-up care between all the specialties involved. The records held include study models, clinical photographs, radiographs and details of audiology and speech and language investigations and assessments, collected according to agreed protocols.

**2.7 Record linkage**

H.L.Dunn of the United States national Bureau of Statistics introduced the term ‘Record Linkage’ in 1946: “Each person in the world creates a Book of Life. This Book starts with birth and ends with death. Record linkage is the name of the process of assembling the pages of this book into a volume” (79).

Record linkage studies are those which bring together information on the same individual from two or more independent sources of data (80). The advent of computerised record linkage has allowed for increased speed, consistency and reliability of results, and the ability to process large volumes of data. Datasets can be anonymised to exclude any patient identifiable information. The simplest form of record linkage is known as ‘deterministic linkage’. This involves using a unique numerical identifier to test agreement on one or more variables, and was employed in this study.

Record linkage may be utilised in a case-control observational study where more than one dataset contain information regarding the same individual, and these can

be matched to corresponding controls. Ideally, all the datasets being considered should undergo quality assessment, as key identifiers may be represented differently between sets and make interpretation difficult. Where the data being studied has multiple dates of reporting, the most appropriate date must be selected, and can prove to be a time-consuming task.

### 2.8 Conclusions from this section

The aim of this first section of background information has been to highlight the complex aetiological basis of OFC and the subsequent range of impacts on the life of the affected individual, combined with the intricate framework of care and support they require.

There is a great need for further research into the genetic and environmental aetiological factors, which may inform future preventative strategies. This research must be based upon accurate data, of which important sources include national and international databases and registries, and may utilise the potentially valuable tool of record linkage.

## 2.9 The Metabolic Syndrome, Diabetes and OFC

### 2.9.1 The Metabolic Syndrome

The Metabolic Syndrome (MetS) has been defined as 'the clustering of various metabolic risk factors that include abdominal obesity, dyslipidaemia, hypertension and hyperglycaemia' (81) (Figure 10, table 4). It is closely associated with increased risk of cardiovascular disease and type 2 diabetes, posing a significant concern for public health.

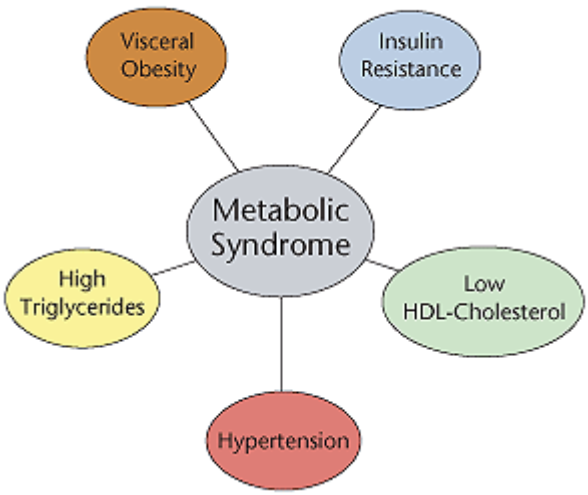



**Figure 10: The Metabolic Syndrome**

Pathophysiologically, the MetS appears to be largely due to insulin resistance and excess fatty acids (82). Therapeutic intervention is aimed at achieving healthy lifestyle changes such as weight loss, healthy eating and physical activity.

**Table 2: Metabolic Syndrome World-wide Definition, adapted from** (83)

| |
|---|
| **CENTRAL OBESITY:**<br><br>Men >40 inch waist circumference<br><br>Women >35 inch waist circumference |
| Plus any two of the following:<br><br>• **RAISED FASTING TRIGLYCERIDES:**<br>≥ 150 mg/dl (1.7 mmol/l)<br>or specific treatment for this lipid abnormality<br><br>• **REDUCED HDL-CHOLESTEROL:**<br>< 40 mg/dl (1.03 mmol/l) in males<br>< 50 mg/dl (1.29 mmol/l) in females<br>or specific treatment for this lipid abnormality<br><br>• **RAISED BLOOD PRESSURE:**<br>Systolic ≥ 130 mmHg<br>or Diastolic ≥ 85 mmHg<br>or treatment of previously diagnosed hypertension<br><br>• **RAISED FASTING PLASMA GLUCOSE:**<br>≥ 100mg/dl (5.6 mmol/l)<br>or previously diagnosed type 2 diabetes |

### 2.9.2 Diabetes

Diabetes mellitus is one of the most prevalent chronic diseases worldwide, with incidence currently increasing; this can be related to changes in lifestyle factors such as reduced physical exercise and a global increase in obesity. Type 1 diabetes occurs due to autoimmune destruction of the insulin-producing cells of the pancreas and is mainly diagnosed in children and young adults. Type 2 diabetes is generally diagnosed in adults over 40; however the number of younger people developing the disease is rising. In type 2 diabetes, insulin production may be insufficient or there may be insulin resistance, which may be related to autoimmunity, single gene mutations and obesity.

Globally, type 2 diabetes is increasing in all age groups and accounts for over 90% of all recorded cases of diabetes (84,85). The prevalence of diabetes increased by 33% in the USA between 1990-1998 (86). Worldwide estimates of diabetes prevalence were 2.8% in 2000 (87), while Shaw et al predict a global prevalence of 7.7%, or 439 million adults, by the year 2030 using data from 216 countries in the United Nations (88).

There are greater numbers of women with diabetes than men, and a worrying increase in the prevalence of type 2 diabetes in adolescents (89). Individuals with type 2 diabetes also commonly posses other risk factors for cardiovascular disease including dyslipidaemia and hypertension. The diagnostic blood glucose levels for diabetes are seen in **table 3**.

Glycosylated haemoglobin (HbA1c) acts as a marker for average plasma glucose concentration. It is a form of haemoglobin formed following exposure to plasma glucose and reflects average plasma glucose over the previous 8 to 12 weeks. It can be performed at any time of the day and does not require any special preparation such as fasting. The WHO recommends An HbA1c of 6.5% is as the cut point for diagnosing diabetes, however a value of less than 6.5% does not exclude diabetes diagnosed using glucose tests.

The use of HbA1c can avoid the problem of day-to-day variability of glucose values, and importantly it avoids the need for the person to fast and to have preceding dietary preparations.

**Table 3: Diagnostic blood glucose levels for diabetes (From WHO)**

| Condition | 2 hour postprandial glucose (mmol/l) | Fasting glucose (mmol/l) | HbA1c (%) |
|---|---|---|---|
| Normal | <7.8 | <6.1 | <6.0 |
| Impaired fasting glycaemia | <7.8 | ≥6.1 & <7.0 | 6.0-6.4 |
| Impaired glucose tolerance | ≥7.8 | <7.0 | 6.0-6.4 |
| Diabetes mellitus | ≥11.1 | ≥7.0 | ≥6.5 |

### 2.9.3 Diabetes in Scotland

The Scottish Diabetes Survey (2011) has reported on data collated from the 14 NHS Boards across Scotland, identifying the current prevalence of diabetes and the progress being made in care provision and outcome. Type 1 and type 2 diabetes are reported separately. Some of the key findings include:

- There were 247,278 people with diagnosed diabetes on local diabetes registers, which represents 4.7% of the population of Scotland
- 88% (217,514) had type 2 diabetes
- 11.4% had type 1 diabetes
- 0.6% had 'other' forms of diabetes
- Of those patients who had a recorded BMI, 36.6% of those with type 1 and 31.7% of those with type 2 diabetes were overweight (BMI 25-30), while 24.5% of those with type 1 and 55.4% of those with type 2 diabetes were obese (BMI >30)

- Of those patients with a recorded blood glucose level (HbA1c), 22% of those with type 1 and 62.1% of those with type 2 diabetes had a result <58 mmol/mol (7.5%), which is the target level reported in previous surveys
- 25.1% (type 1) and 18.5% (type 2) were current smokers (90)

The results of this survey clearly show that diabetes is a significant and common problem for the health of the Scottish population. High levels of obesity must play an important role in the development of the current situation with respect to high prevalence of type 1 and type 2 diabetes.

### 2.9.4 Gestational diabetes

Gestational diabetes mellitus (GDM) can be defined as ‘glucose intolerance of various degrees that is first detected during pregnancy’ or ‘any degree of glucose intolerance with onset or first recognition during pregnancy’ (91,92). The White classification distinguishes between new-onset GDM (Type A) and pregestational (undiagnosed) diabetes (Type B) (93). GDM is diagnosed where insulin resistance continues beyond 24-28 weeks of pregnancy. Between 3-10% of pregnancies are affected by GDM (101).

In the UK, a diagnosis of GDM is made following a non-challenge blood glucose test where the plasma glucose level is found to be higher than 126 mg/dl (7.0 mmol/l) after fasting.

Insulin resistance normally increases during pregnancy to a certain extent, and by the third trimester these levels may be equivalent to those seen in type 2 diabetes. However pregnant women with GDM have greater levels of insulin resistance than

normal pregnant women. This may be due to the effects of pregnancy hormones such as cortisol and progesterone produced by the placenta and also possibly increased maternal adiposity. This raised insulin resistance cannot be compensated for by the normal increase in insulin secretion by the pancreatic β cells, as these have been found to have defective function (94).

Following delivery, women with GDM have greater insulin resistance than normal women, and many (35-60% reported) will progress to the development of diabetes over time (95).

The effects of GDM on the developing foetus are still not wholly understood. Glucose transporters 1 and 3 (GLUT1 and GLUT3) protein membrane carriers facilitate the diffusion of glucose across the placenta and embryonic cell membranes during critical periods of embryonic development. In untreated GDM the developing foetus is exposed to consistently higher glucose levels than normal. Foetal insulin levels rise accordingly and stimulate excessive growth and macrosomia. Embryonic cells suffer from increased metabolic load, leading to increased formation of reactive oxygen and oxidative stress. This may result in impaired embryonic gene expression, followed by apoptosis or disturbed organogenesis (96). Following birth insulin production remains high, and the newborn is therefore susceptible to hypoglycaemia.

### 2.9.5 Maternal diabetes and congenital anomalies

A substantial amount of epidemiological research implicates maternal diabetes as a highly significant risk factor for birth defects, with both *in vivo* and *in vitro* studies have identified hyperglycaemia as factor that may induce congenital malformations. Maternal obesity and high pre-pregnancy weight are also associated with the risk of congenital anomalies (96).

The descriptive study reported by the Confidential Enquiry into Maternal and Child Health of pregnant women with type 1 and 2 diabetes in England, Wales and Northern Ireland (84) looked at rates of Perinatal mortality, still birth, neonatal mortality and congenital anomalies. It found that of a total of 620,830 births in these countries in 2002, 2359 were born to women with pregestational type 1 and 2 diabetes (0.38%). The median pre-pregnancy HbA1c value was 7.9%, and women with type 1 diabetes had higher levels of HbA1c throughout pregnancy than those with type 2. A higher pre-pregnancy HbA1c level of 8.35% was seen in women who had a baby with major congenital malformations. No difference was identified in perinatal mortality rates of babies of women with type 1 or type 2 diabetes.

The prevalence of major congenital anomalies was 41.8 per 1000 births, with a three-fold increase in anomalies of the circulatory system and neural tube defects. Of the reported 2359 babies born to women with diabetes, one (0.042%) had CL(P) and three (0.13%) had CP.

The possible link between diabetes and raised maternal blood glucose levels with congenital anomalies including OFC has been the subject of numerous previous studies. Vallance-Owen et al (1967) studied 34 mothers who had babies with CL(P) and found them to have increased insulin resistance related to diabetes (97).

Navarette et al (1970) studied 349 women who had babies with congenital malformations and found a ‘high and increasing frequency of known diabetes’ over 0, 12 and 25 years. They hypothesised that there was a definite link between maternal glucose metabolic disorder and congenital malformations. None of the case mothers had abnormal fasting glucose during their pregnancy with the malformation, leading the authors to conclude that “a search for a latent glucose metabolic disorder in mothers bearing a malformed baby might make a useful contribution to the prevention of human congenital malformations.” (98).

As part of the Atlanta Birth Defects Case-Control Study, Becarra et al looked at 4929 births with major congenital anomalies and 3029 matched non-malformed babies. The relative risk for major congenital anomalies with insulin-dependent diabetes was 7.9 compared to infants from non-diabetic mothers. This study may have been limited by its retrospective design and an underestimation of diabetes orevalence. Diamond (1996) found congenital malformations in 13% of 199 babies born to mothers with type1 diabetes, however this did not include CL(P) (99).

Towner et al studied 332 consecutive infants born to mothers with type 2 diabetes over a 6 year period. 16.9% had one or more congenital anomaly. Maternal HbA1c level (mean 9.5 ± 0.4%) was directly associated with major malformations. Women who had a younger age at onset of diabetes were also found to have an increased risk (100). This study utilised prospectively collected data and stepwise regression analysis.

In a study of mothers with established and gestational diabetes (GDM), Janssen et al found that infants born to mothers with established diabetes are more likely to have congenital anomalies than infants born to non-diabetic mothers (prevalence odds 4.0), however only a slightly higher prevalence of congenital anomalies were

found in the babies of mothers with GDM. They recommended screening for impaired glucose tolerance (IGT) to identify women with undiagnosed abnormalities of glucose metabolism, and are thus at increased risk of delivering an infant with malformations (101). Schaefer-Graf et al investigated patterns of congenital anomalies in relation to maternal fasting glucose levels in type 2 diabetes and GDM. In this study of 3764 pregnancies with GDM and 416 with type 2 diabetes, increased hyperglycaemia was found to be positively related to congenital anomalies; however they did not compare these cases to a control group (102).

Macintosh et al used data from the Confidential Enquiry into Maternal and Child Health (CEMACH 2002-2003) to study rates of congenital anomalies in babies born to women with types 1 and 2 diabetes. They reported high rates of malformations, although there we no babies with CL(P) and only 2 of 2400 babies had isolated CP (103). Farrell et al carried out a prospective study over fifteen years and found that women with GDM who were likely to have had pre-existing undiagnosed type 2 diabetes had the same rate of congenital anomalies in their offspring as women with established type 1 or 2 diabetes. While in the remainder of the GDM group, the rate did not differ from that seen in non-diabetic mothers. This study may have suffered from under-reporting of pre-existing diabetes and a lack of control mothers (104).

Loeken carried out a study using a mouse model and identified excess glucose metabolism by the embryo resulting from maternal hyperglycaemia, leading to oxidative stress with impaired embryonic gene expression and neural tube defects (105). This may not however be directly relatable to these conditions in humans.

Aberg et al carried out a study using the Swedish Medical Birth registry from 1987-1997. In mothers with pre-existing diabetes the total malformation rate was 9.5% and included OFC, while the rate for mothers with GDM was similar to the rate in

the general population at 5.7%. They concluded that within the group of mothers with GDM there may be a subgroup with an increased risk of diabetic embryopathy, which may be due to undiagnosed pre-existing diabetes (106). This study was strengthened by the availability of a large birth registry covering over one million births.

In a study of mothers with type 1 diabetes in Scotland, Penney et al identified the rate of congenital anomalies as 60 per 1000 total births, however there were no babies born with CL(P) from a sample of 273 pregnancies (107). Strengths of this study were its prospective design and the inclusion of pregnancies ending in miscarriage and abortion, as well as delivery.

### 2.9.6 Maternal diabetes and OFC

In the first study to specifically investigate the association between maternal diabetes and OFC, Spilson et al (2001) conducted a population based case-control study using the 1996 National Centre for Health Statistics United States Natality Database. Diabetic mothers were 1.352 times more likely than non-diabetic mothers to have a newborn with CL(P) (95% CI, 1.004-1.821; $P<0.05$). They did not differentiate between forms of diabetes or separately analyse different types of cleft, and the authors suggested that future should also include information on the modality of treatment for diabetes (3).

Carinci et al studied 126 infants born with non-syndromic OFC in Southern Italy , and using univariate analysis, found that familial diabetes was associated more often with isolated CP than other forms ($P=0.0014$), however the sample was reasonably small and no control group was included (108).

Evers et al concluded that even near-optimal maternal glucose control is not adequate in the prevention of congenital anomalies including OFC, as their incidence in planned pregnancies is significantly lower than in unplanned pregnancies (109). Suhonen et al also stressed that 'normoglycaemia should be strived for during early pregnancy' as even slightly raised HbA1c in early pregnancy was found to increase the risk of birth defects in the Southern Finnish population (110). A systematic review by Wahabi et al concluded that preconception care is effective in reducing diabetes related congenital anomalies and Mossey et al found that in a UK-based study planned pregnancies were associated with a lower risk of OFC (111,112).

### 2.9.7 Maternal overweight and congenital anomalies

Obesity is an important and growing concern to public Health across the globe. Worldwide, the number of overweight (body mass index [BMI] 25-30, calculated as weight in kilograms divided by height in metres squared) and obese (BMI ≥30) adults is expected to be 2.3 billion and over 700 million respectively, by 2015 (113). In the USA, 50% of women aged 15-49 are currently overweight or obese (114), while in Australia 22.6% of 25-34 year old women were overweight, and 12.4% obese, in 2000 (115).

Pre-pregnancy maternal obesity has significant health implications for both mothers and their babies, including the incidence of congenital anomalies. Abdominal adipose tissue accumulation associated with diabetic pathogenesis may be the underlying mechanism for the link between obesity and such birth defects. Interestingly, Shaw and Carmichael found that in a Californian population there was an association between diabetogenic metabolic pathogenesis and obese women

with weight gain around the waist, but not for women with weight gain on the hips (116).

In a study of almost 23,000 pregnant women, Moore et al found that individually, obese women (BMI≥30) and women with pre-existing diabetes or GDM had no excess overall risk of having a baby with a major birth defect (prevalence ratios 0.95 and 0.98 respectively) however where women were both diabetic and obese, there was a 3.1 times greater risk of major congenital anomalies. Therefore this suggests a synergistic interaction between diabetes and obesity in the pathogenesis of congenital anomalies (117).

Martinez-Frias et al carried out a study using data from the Spanish Collaborative Study of Congenital Malformations (ECEMC) and found that in a group of mothers with GDM, obesity (BMI≥30) was associated with a significant increase in the risk of cardiovascular defects when compared to non-diabetic mothers. In mothers with normal glucose tolerance (NGT) pre-gestational BMI was not associated with congenital anomalies (118). This study utilised pre-pregnancy BMI which was ascertained through post-partum interview and may not be reliable. There was slightly less than one control mother per case, although the sample size was large, with over 30 000 in each group.

Waller et al found a ‘weak to moderate’ association of maternal obesity with some birth defects including cleft palate in a study of over 10,000 women, excluding those with pre-existing diabetes (119). Biggio Jr et al concluded that maternal weight alone is not associated with an increased risk of congenital anomalies; however pre-gestational diabetes is (120). This cross-sectional study included almost 42 000 births, and where information regarding maternal BMI was unavailable, those weighing over 200lbs at their first pre-natal visit were defined as obese.

Parker et al looked at the association between high maternal dietary glycaemic index (DGI) and birth defects, and excluded women with pre-existing diabetes and GDM. They found an association between high DGI (based on a questionnaire) and CLP (adjusted OR, 1.23). They also describe evidence of a synergistic effect of high DGI and obesity in selected birth defects, suggesting that while high DGI is associated with an increased risk of several birth defects, obesity combined with high DGI may increase this risk further (121).

### 2.9.8 Maternal overweight and OFC

A study by Cedergren and Kallen found a positive association between maternal obesity (BMI≥29) in early pregnancy and OFC (OR, 1.30) and that this risk was higher where clefting was associated with other major malformations (122). This study included a very large control group and there was a high level of completeness of reporting of BMI data in the registries utilised.

In another Swedish study of 220,328 mothers, Villamor et al found that among women whose second-pregnancy BMI was ≥3 units higher than their first-pregnancy BMI, the adjusted risk of isolated CP was 2.3 times higher, however increased BMI was not related to risk of CL. Long interpregnancy intervals also appeared to be associated with increased risk of CP (123). The authors found that almost three quarters of the pregnancies documented in the Swedish Birth registry were found to have complete data regarding maternal height and weight early in pregnancy.

In a case-control study in Western Australia, Oddy et al concluded that mothers with pre-pregnancy obesity (BMI≥30) had a two-fold increased odds of having a baby with OFC (115). Maternal pre-pregnancy weight and height were self-reported

through a questionnaire. Live births, still births and terminations of pregnancy were included in this study.

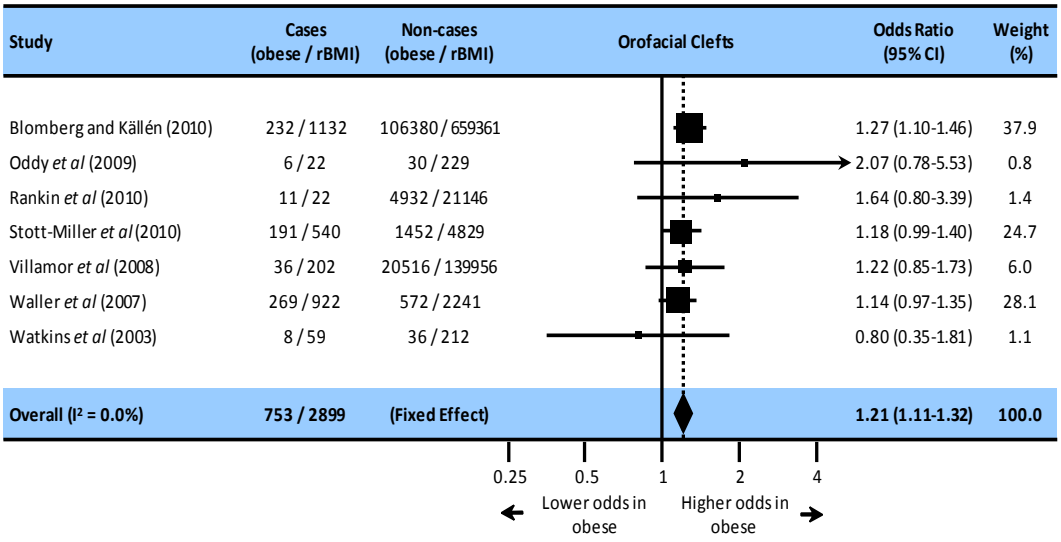
Stott-Miller et al carried out a population-based case-control study of infants with OFC (n=2153) and control infants (n=18070). They found that obese (BMI≥30) mothers had a 'small increased risk' of non-syndromic OFC in offspring compared to women of healthy BMI (adjusted OR, 1.26). Overweight (BMI≥25 and <30) mothers had an elevated odds of CL/P but not of isolated CP. Additionally, odds of non-syndromic CL/P is 23% higher for each 10-point increase in maternal BMI (4). The authors reported a potentially significant limitation of a considerable amount of missing data for maternal BMI and pre-pregnancy weight. However they did carry out a secondary analysis with imputation of missing data in order to reduce the effect of bias.

In a study of over 1 million births in Sweden, Blomberg and Kallen found that maternal pre-pregnancy morbid obesity (BMI≥40) is associated with OFC (OR 1.90) (124). The availability of data regarding maternal BMI was high, with maternal pre-pregnancy weight and height reported for 85%. An advantage of this study was accessibility to a large-scale population register, increasing the statistical power of reported results.

A recent study by Marengo et al found the risk of birth defects including CL(P) to be 'substantially' increased in obese mothers in a Texan population (125). This was also a large scale study, covering around 1.6 million births and utilising self-reported maternal pre-pregnancy height and weight.

A summary of the meta-analysis by Stothard et al (113) is illustrated in figure 11. This indicates the higher risk of OFC occurring in the offspring of obese mothers.

**Figure 11: Meta-analysis of maternal obesity and OFC, adapted from (Stothard et al, 2009 (113))**




### 2.9.9 Summary and conclusions from this section

The number of women with diabetes and who are overweight or obese is increasing worldwide, with an increased risk of offspring having CL(P) and numerous other congenital anomalies seen to be positively associated with both of these factors.

Mothers with established diabetes may be at greater risk than those with gestational diabetes mellitus. The mechanism implicated in causing such birth defects may be related to maternal hyperglycaemia and impaired glucose metabolism during pregnancy leading to oxidative stress within the developing embryo. Because gestational diabetes develops towards the latter stages of pregnancy, while OFC develops within the first two months, the discrepancies in results between GDM and

established diabetes may not disprove the theory that impaired glucose metabolism is linked to development of OFC (4) .

It would appear that both maternal hyperglycaemia and obesity are risk factors for congenital anomalies including OFC individually; however the relative risk may be increased when they are combined together synergistically. Different patterns of maternal weight gain may affect the risk of birth defects including OFC. There may also be aetiological differences related to CP and CL.

The studies detailed have provided valuable evidence and insight into the roles of maternal diabetes and obesity in the development of congenital anomalies including OFC, and their findings must be supported and strengthened by further collaborative studies to elucidate both environmental and genetic risk factors contributing to OFC and the interactions between them. The positive role of pre-pregnancy planning including the management of hyperglycaemia and maternal weight should also be the focus of future work toward preventive strategies.

**2.10 Relevance to the current project**

The significant body of work suggesting an aetiological role of both maternal diabetes and obesity in the development of OFC provides incentive to further investigate this link in our own population. The valuable resource of the CLEFTSiS database and other registries held within NHS Tayside provide scope for a linkage study, which may inform future Scotland-wide or even further reaching studies of these possible associations. This will have an informative effect on possible prevention strategies for OFC.

# CHAPTER THREE: AIMS AND HYPOTHESES

## 3.1 Aim

The aim of this study was to investigate the association between oral cleft in children and diabetes and obesity in their mothers in the Tayside population.

## 3.2 Longer term aims

This study was designed as a pilot to a potential larger study (a) in certain European countries already identified through the European Science Foundation (ESF) and (b) In India where, via an NIH planning grant the following hypotheses will be tested: (1) Impaired glucose tolerance and diabetes are each associated with risk of oral clefts; (2) Impaired 1-CM and impaired glucose tolerance or diabetes interact to increase risk of oral clefts.

## 3.3 Null hypotheses

- Maternal diabetes confers no increase in the risk of children being born with cleft lip and/or cleft palate in the population of Tayside
- Maternal obesity confers no increase in the risk of children being born with cleft lip and/or cleft palate in the population of Tayside
- The information held within the CLEFSiS dataset and other maternal datasets within NHS Tayside has a high level of completeness which is required for accurate assessment and linkage

The amount and quality of the data must allow for meaningful analysis between the case and control mothers, and ideally include sufficient information regarding

confounding factors in order to separate their influence from that of the independent variables.

# CHAPTER FOUR: MATERIALS AND METHODS

The unique circumstances within Tayside, featuring the existence of the Health Informatics Centre with access to both the CLEFTSiS database and other datasets of maternal information within NHS Tayside, enabled a record linkage study to be carried out.

This study involved no patient-identifiable information, as all data were linked using the Community Health Index (CHI) number. Therefore no ethical approval or Caldicott Guardian approval was required. The study was sponsored by Tenovus Scotland.

A sample size calculation was not performed as this was a pilot study, data for all case mothers within the available date range was included and the sample size would be limited to this number of mothers regardless of whether a calculation was carried out.

## 4.1 The Health Informatics Centre

The Health Informatics Centre (HIC) is a University research support unit operating within the college of Medicine, Dentistry and Nursing at the University of Dundee, in collaboration with NHS Tayside. HIC provides data users with linked anonymised information from population based health datasets, mainly from the NHS and the University of Dundee. Most datasets already have patient unique identifiers (CHI numbers) however HIC additionally adds unique identifiers to datasets that lack them.

Access to the anonymised individual data for analysis is provided within the HIC-based ‘Safe Haven’ environment. Data users log on remotely to a secure server located within HIC in order to access data and perform analyses. Data cannot be copied or removed from the secure central server.

The remote-access Safe Haven utilises the Citrix Xen Desktop secure environment. This is widely used by the military and government to provide secure access to information. Data are not released externally to data users, rather they are placed on a server at HIC within a secure IT environment, and the data user is given secure remote access for analysis.

To enable an output data file to be removed from the Safe Haven, the data user moves the file to the output directory within their Safe Haven personal directory. The output file(s) are reviewed by a HIC Data Analyst between 9 -11 am the next working day, and once verified as not containing any patient-identifiable information, they are then emailed to the data user.

### 4.2 HIC Data Requirement

For this project, HIC initially identified a list of babies with OFC from the CLEFTSiS database born between 1990-2010, within the Tayside area. These were then given an anonymised patient identifier (PROCHI) based on their CHI number.

#### Exclusion of records

Syndromic and atypical clefts were excluded from the cohort sample. This was due to the believed differences in aetiology between non-syndromic OFC and syndromic and atypical clefts. The exclusion criteria were applied by removing any data

regarding babies with a positive entry in the column ‘cleft syndrome name’ within the Microsoft excel database.

**Inclusion criteria**

All babies born with non-syndromic OFC recorded within the CLEFTSiS database, born between 1990 and 2010 within Tayside, where no exclusion criteria applied.

### 4.3 Identification of maternal data

HIC identified the mothers of the babies selected from CLEFTSiS through the SMR02 database. SMR02 is a dataset of maternity inpatient and day cases. It contains data covering the whole of Scotland and includes information regarding mother and baby characteristics, birth weight, gestational age, mode of delivery and outcome of pregnancy. The dataset contains the mother and baby’s CHI numbers.

### 4.4 Maternal data supplied

The following datasets were provided by HIC regarding the cohort of mothers of babies with OFC:

SMR02

SCI-DC – Demography

Body Mass Index

HbA1c (blood glucose level) – taken in the first trimester of pregnancy (as per previous studies, (100,110)

### 4.4.1 SCI-DC

The Scottish Core Information – Diabetes Collaboration (SCI-DC) contains the Scottish Diabetes Core Dataset as part of a national framework with the aim of supporting regional Managed Clinical Networks for diabetes by providing IT support, clinical information and data for national and local audit programmes. The datasets are developed and maintained by the SCI-DC Development Team based in the Clinical Technology Centre at Ninewells Hospital, Dundee.

The following datasets were supplied:

**Table 4: SCI-DC Demography**

| Field | Description |
|---|---|
| PROCHI | 10 digit anonymised CHI generated by HIC. The first 3 digits are character, the last 7 are integers e.g. abc1234567 |
| Anon_date_of_birth | Anonymised date of birth |
| Date_in | Date a person came into the health board region |
| Date_out | Date a person left this health board |
| TYPE_DM | Code identifying type of diabetes e.g. 1=Type 1 |
| TYPE_DM_Descriptor | Descriptive text of type of diabetes e.g. Type 1 |
| DtDiag | Date person was diagnosed with diabetes |

### Table 5: SCI-DC HbA1c

| Field | Description |
|---|---|
| PROCHI | 10 digit anonymised CHI generated by HIC. The first 3 digits are character, the last 7 are integers e.g. abc1234567 |
| Test_date | When HbA1c was performed |
| Result_DCCT | DCCT result (%) |
| Test ID | Indicates type of test e.g. 9=DCCT |
| DataSource_ID | ID of where test was performed |
| DataSource_Description | Description of where the test was performed |

### Table 6: SCI-DC BMI

| Field | Description |
|---|---|
| PROCHI | 10 digit anonymised CHI generated by HIC. The first 3 digits are character, the last 7 are integers e.g. abc1234567 |
| Date | When BMI was taken |
| Height | Patient height (metres) |
| Weight | Patient weight (kgs) |
| BMI | Calculated from weight/height. Where this is blank, HIC will calculate it from the supplied heights/weights |
| DataSource_ID | ID of where test was performed |
| DataSource_Description | Description of where the test was performed |

### 4.5 Level of deprivation

The level of deprivation of the mothers was identified from the demography datasets provided using the Scottish Index of Multiple Deprivation (SIMD). This is an index linked to postcode and is a key tool for identifying area concentrations of deprivation. It is used for a wide range of purposes including as a statistical classification and as an indicator to target resources and policies at small areas. It also feeds into work looking at health inequalities across Scotland (Scottish Government).

The SIMD covers the whole of Scotland, which is broken down into 6505 datazones. These datazones are grouped into five categories, which range from 1 being 'most deprived' to 5 being 'least deprived'.

Figures 11, 12 and 13 show the population structure of each Tayside locality by SIMD category. The charts demonstrate that the Dundee has the largest deprived population across Tayside's three local authority areas. (NHS Tayside)

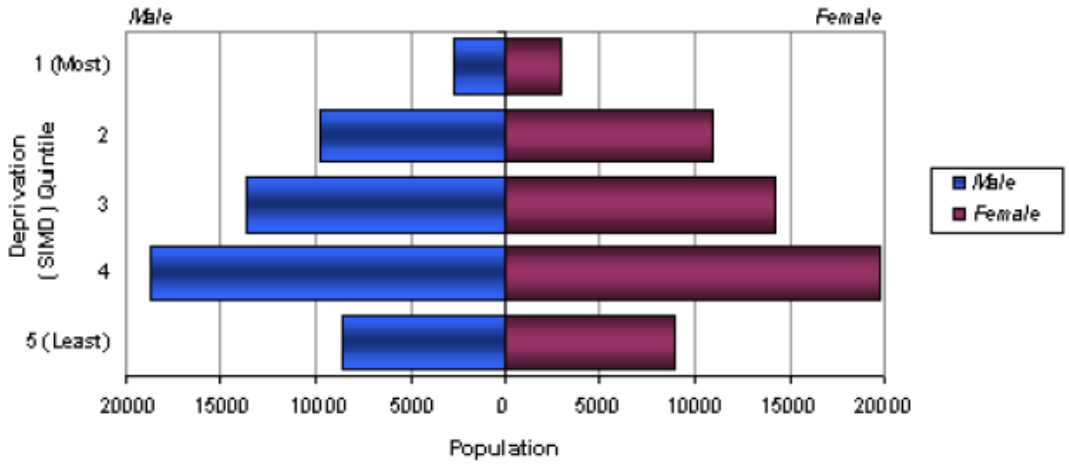



**Figure 12: Deprivation profile: Angus**

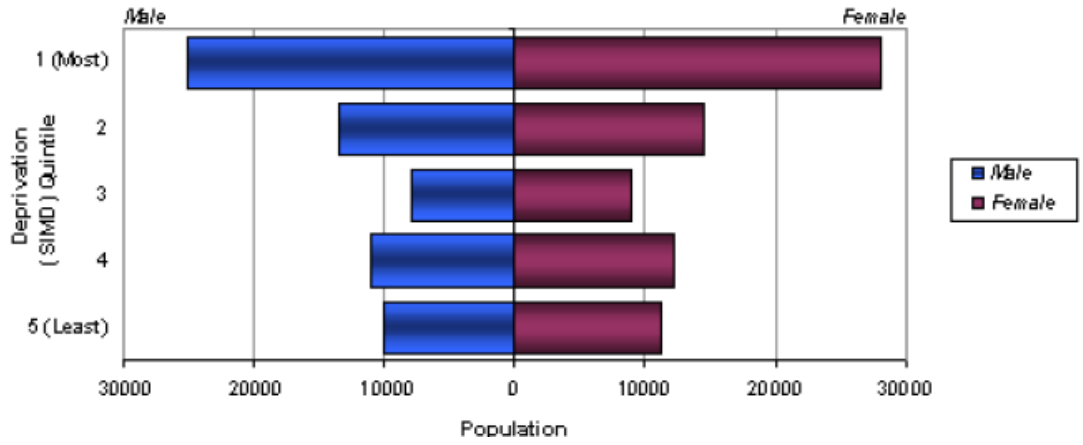



**Figure 13: Deprivation profile: Dundee City**

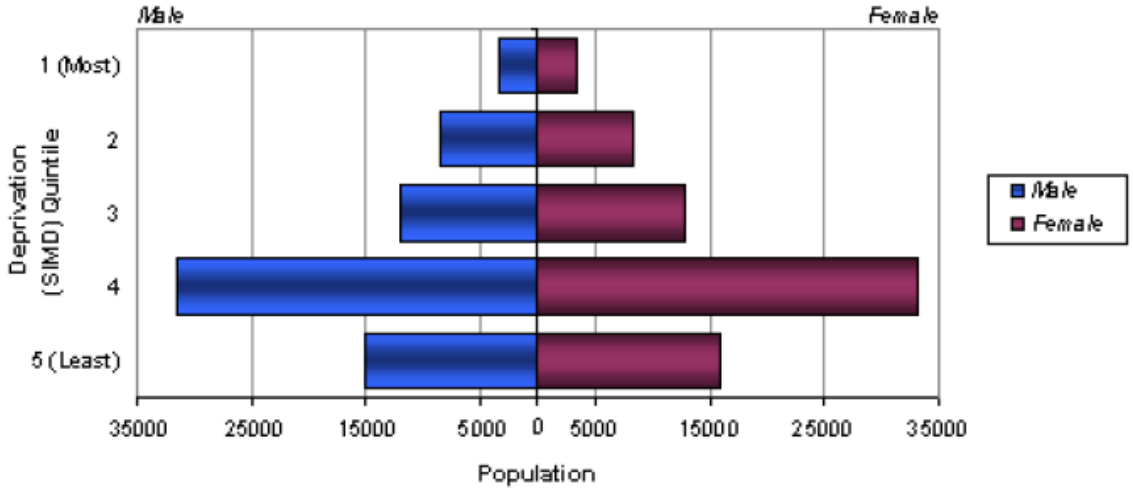



**Figure 14: Deprivation profile: Perth and Kinross**

### 4.6 Control sample

Four control mothers for each case mother were randomly selected from the SMR02 dataset by HIC. It was felt that this would increase the statistical power of the results. These mothers had a date of delivery +/- 3 months from the case births, and were also matched on age at delivery. All controls were resident in Tayside at the time of the birth and had been for at least one year prior to the birth.

The control group consisted of 564 mothers, based upon an original group of 141 case mothers prior to only the unique patients being isolated. The datasets provided regarding the control mothers were the same as for the cases, but supplied within separate files.

### 4.7 Data management

Datasets were supplied by HIC in Microsoft Excel spreadsheet format (Microsoft, Redmond, California, USA). Where datasets included more than one test result for the same mother, they were individually searched and the date most closely corresponding with the date of delivery extracted. Statistical analysis was then performed using IBM SPSS Statistics software version 21 (IBM corp., NY, USA) after the files had been converted to this format.

### 4.8 Statistical analysis

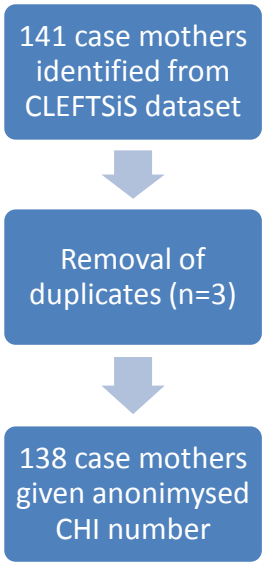
All statistical analyses were performed using the SPSS programme. This was following advice and assistance from a medical statistician.

Comparison between the proportion of mothers with known diabetes within the case and control groups was made using the Mantel-Haenszel Common Odds Ratio Estimate following data entry into a 2x2 table. This assumed that the population of the sample groups had a normal distribution and assessed categorical data (diabetes = either ‘yes’ or ‘no’).

The independent samples t-test assuming unequal sample size and unequal variance was utilised to analyse the numerical data relating to the mean weight and blood glucose levels (HbA1c) of the case and control mothers.

# CHAPTER FIVE: RESULTS

A total of 138 case mothers and 564 control mothers were included in this study. These were identified following elimination of any duplicates and supplied by HIC. All data were regarding live births as abortions are not reported in the CLEFTSiS dataset.




## 5.1 Age of mothers

The case and control mothers were matched on age at delivery as well as date of delivery. The demographics are demonstrated in table 9:

**Table 7: Age of mothers at delivery**

| | Minimum age | Maximum age | Mean age | Standard deviation |
|---|---|---|---|---|
| Cases | 18 | 45 | 28.9 | 5.63 |
| Controls | 18 | 44 | 28.8 | 5.76 |

### 5.2 Level of deprivation

The level of deprivation of the mothers linked to postcode using the Scottish Index of Multiple Deprivation (SIMD) was identified from the SMR02 dataset. A SIMD score was found to be recorded for 129/138 case mothers and 5454/564 control mothers.

**Table 8: Frequency and percentage of SIMD scores: case mothers**

| SIMD score | Frequency | % |
|---|---|---|
| 1 | 27 | 20.9 |
| 2 | 15 | 11.6 |
| 3 | 22 | 17.1 |
| 4 | 41 | 31.8 |
| 5 | 24 | 18.6 |
| Total | 129 | 100 |

**Table 9: Frequency and percentage of SIMD scores: control mothers**

| SIMD score | Frequency | % |
|---|---|---|
| 1 | 93 | 17.1 |
| 2 | 84 | 15.4 |
| 3 | 100 | 18.3 |
| 4 | 157 | 28.8 |
| 5 | 111 | 20.4 |
| Total | 545 | 100 |

**Table 10: SIMD scores: case and control mothers**

| SIMD score | Cases | Controls | Total |
|---|---|---|---|
| 1 | 27 | 93 | 120 |
| 2 | 15 | 84 | 99 |
| 3 | 22 | 100 | 122 |
| 4 | 41 | 157 | 198 |
| 5 | 24 | 111 | 135 |
| Total | 129 | 545 | 674 |

Chi-squared test significance: 0.828

### 5.3 Types of clefting

The phenotype of OFC affecting the babies of the mothers in the case group was recorded.

**Table 11: Frequencies of OFC phenotype**

| Phenotype | Frequency | % |
|---|---|---|
| **BCL** | 3 | 2.2 |
| **BCLP** | 6 | 4.4 |
| **UCL** | 37 | 26.3 |
| **UCLP** | 28 | 20.6 |
| **CP** | 63 | 45.8 |
| **CL** | 1 | 0.7 |
| **Total** | 138 | 100 |

One case was recorded as having ‘CL’ with no indication of laterality.

Of the 63 cases with CP only recorded, 38 (60%) were female. Of the 75 cases recorded as having CL/ P, 32 (43%) were female.

### 5.4 Comparison of prevalence of diabetes within the case and control groups

Within the group of 138 case mothers, 4 were identified as having diabetes from the SCI-DC dataset. Within the control group of 564 mothers, 13 were identified as being diabetic.

Only 1 of the 4 diabetic case mothers and 2 of the 13 diabetic control mothers had a data entry for date of diagnosis with diabetes.

**Table 12: Prevalence and type of diabetes affecting case and control mothers**

| | Type of diabetes | | | **Total** |
|---|---|---|---|---|
| | Type 1 | Type 2 | Other | |
| **Cases** | 2 | 1 | 1 | **4** |
| **Controls** | 6 | 6 | 1 | **13** |
| Total | 8 | 7 | 2 | 17 |

**Table 13: 2x2 table of diabetes prevalence**

| | Diabetic | Non-diabetic | Total |
|---|---|---|---|
| Cases | 4 | 134 | 138 |
| Controls | 13 | 551 | 564 |
| Total | 17 | 685 | 702 |

Mantel-Haenszel common odds ratio estimate: 1.265

95% Confidence interval of common odds ratio: 0.406-3.942

**Table 14: Chi-squared test comparing prevalence of diabetes**

| N of diabetic case mothers | N of diabetic control mothers | Significance | 95% confidence interval | Odds ratio |
|---|---|---|---|---|
| 4 | 13 | 0.6305 | 0.3611, 3.903 | 1.304 |

Significance was set at $p < 0.05$

### 5.5 Comparison of blood glucose levels (HbA1c)

Within the SCI-DC datasets, measurements of glycosylated haemoglobin had been recorded as standardised units against the 1993 Diabetes Control and Complications trial (DCCT). The DCCT unit is recorded as HbA1c %. The DCCT value taken in the first trimester of pregnancy for each mother was identified and the mean value calculated.

**Table 15: Mean HbA1c values of diabetic case and control mothers**

| | Number | Minimum HbA1c(%) | Maximum HbA1c(%) | Mean HbA1c(%) | Standard deviation |
|---|---|---|---|---|---|
| Cases | 4 | 6.5 | 11.0 | 7.975 | 2.069 |
| Controls | 13 | 5.2 | 9.6 | 6.846 | 1.475 |

**Table 16: Independent samples t-test for equity of mean HbA1c**

| Mean HbA1c cases | Mean HbA1c controls | Significance | 95% Confidence interval |
|---|---|---|---|
| 7.975% | 6.846% | 0.368 | -4.222, 1.964 |

### 5.6 Comparison of maternal BMI

There were found to be large deficiencies with data entries in the SCI-DC BMI dataset, with none of the diabetic case mothers having an entry, and only one of the diabetic control mothers having a recorded BMI, which was not close in date to that of delivery. Therefore maternal weight recorded at the date closest to the date of delivery was identified from the SMR02 dataset and compared between case and control mothers. There was insufficient data regarding height and weight to calculate BMI.

Weight (kg) had been recorded at the time of admission for delivery for 21/138 case mothers and 89/564 control mothers.

**Table 17: Mean weight**

| | Number | Minimun weight (kg) | Maximum weight (kg) | Mean weight (kg) | Standard deviation |
|---|---|---|---|---|---|
| Cases | 21 | 47 | 132 | 71.05 | 20.996 |
| Controls | 89 | 49 | 138 | 75.08 | 20.394 |

**Table 18: Independent samples t-test for equity of mean weight**

| Mean weight case mothers | Mean weight control mothers | Significance | 95% Confidence interval |
|---|---|---|---|
| 71.05 kg | 75.08 kg | 0.418 | -14.378, 6.316 |

# CHAPTER SIX: DISCUSSION

## 6.1 Sample numbers, completeness of datasets and confounding factors

Data regarding a total of 138 case mothers and 564 control mothers were available for analysis; however the numbers of mothers recorded as being diabetic was low at 4/138 and 13/564. This prevented further analysis of the differing categories of diabetes between groups.

It was discovered during examination of the CLEFTSiS, SMR02 and SCI-DC datasets that information regarding the mothers in the sample was frequently missing. There was insufficient data to calculate and analyse maternal BMI, therefore maternal weight was analysed instead. This is not the ideal measurement to investigate associations with maternal obesity.

The smoking and alcohol status of the mothers in the CLEFTSiS dataset was recorded in 62 of 138 cases (45%), while in the SMR02 dataset smoking status was only recorded for 98 of the 564 control mothers (17%). It was therefore not possible to analyse these potential confounding factors.

## 6.2 Maternal age at delivery

As case and control mothers had been matched on age at delivery, it was unsurprising that their mean ages at delivery were very similar at 28.9 and 28.8 years respectively. The case mothers' age at delivery ranged from 18 to 45 years.

### 6.3 Maternal level of deprivation

The SIMD scores, linked to postcode, of the case and control mothers at the time of admission for delivery were not significantly different ($p$ = 0.828). The highest proportion of both groups were SIMD score 4 (31.8% case mothers and 28.8% of control mothers).

Both groups had similar numbers in each SIMD category, reflecting the general distribution of population in Tayside, with most females in the Angus and Perth areas in SIMD category 4, while the slightly raised number of both case and control mothers in SIMD category 1 is reflective of the Dundee City population.

### 6.4 Types of clefting

Within the sample group of babies born with OFC in Tayside, 45.8% were documented as having isolated CP. This is higher than the 20-25% reported in European and US studies (1). The perceived differences in aetiology between CP and CL/P may therefore be relevant in the Tayside population.

Isolated CL made up 29% of the sample group, which is comparable to the 20-25% seen in these larger studies. Cases of CLP made up 25% of the sample, which is lower than the 30-35% reported there.

Within the CLP group in this study, 82% had a unilateral cleft and 18% of clefting was bilateral, which is very similar to the 80% and 20% reported in the larger studies.

Of the 63 recorded cases with CP only, 38 (60%) were female, which is in accordance with the reported female predominance in CP (86). Of the 75 cases recorded as having CL/ P, 32 (43%) were female, which is higher than the 2:1 male:female ratio generally found in white populations (12).

### 6.5 Prevalence of diabetes

Within the control group, 2.3% (13/564) of mothers were documented as having diabetes. This is reduced in comparison to the 4.7% of the Scottish and 5% of the Tayside populations with diabetes (126). This may be attributable to the relatively young age of these women at delivery compared to the increasing age of the general population and the morbidities associated with this including diabetes.

2.9% of the case mothers were diabetic, again low in comparison to regional and national figures. This figure was similar to the 3.6% found by Spilson et al in a larger US population sample (3), therefore this Tayside sample may well be representative of both national and international populations.

There was no significant difference between the prevalence of diabetes in the case and control groups (OR 1.265, 95% Confidence interval: 0.406, 3.942, $p = 0.6305$), therefore a link between maternal diabetes and OFC in their offspring could not be identified within the Tayside population. The sample size was, however, limited and the potential for selection bias exists as it cannot be guaranteed that all patients diagnosed with diabetes are registered in the SCI-DC dataset.

There was also poor reporting of the date of diagnosis with diabetes within the SCI-DC dataset. Only 1 of the 4 case mothers with diabetes and 2 of the 13 control

mothers had a positive entry for date of diagnosis. This prevented further analysis based upon duration of diabetes history.

### 6.6 Comparison of blood glucose levels (HbA1c)

There was no significant difference between the mean HbA1c of the case and control mothers who were recorded as being diabetic in the SCI-DC dataset. Both were elevated at 7.975% and 6.846% respectively, in comparison to the healthy reference range of 4-5.9%.

Suhonen et al found that even a slightly elevated HbA1c of 6.8% in women was linked to a relative risk of 3.0 for major foetal malformations (110), while in a study by Towner et al, mothers who had offspring with major congenital malformations were found to have a mean HbA1c of 9.5% (100).

Although no difference was identified between the groups in this study, it seems that in light of the increased risk of congenital malformations associated with raised HbA1c in other studies, an important role for pregnancy planning and the achievement of normoglycaemia must be emphasised.

### 6.7 Comparison of maternal weight

The original intention in this study had been to assess any potential difference in body mass index (BMI) between the case and control mothers in order to ascertain whether there was a link between maternal overweight/obesity and OFC.

Unfortunately data regarding maternal weight and particularly height was poorly reported and therefore was insufficient to calculate BMI. Maternal weight recorded

as near as possible to the date of delivery was identified from the SMR02 dataset. This is not an ideal indicator of the mother's overweight/obesity status as it does not take account of height, unlike BMI.

There was no significant difference in mean weight between the case (71.05 kg) and control (75.08 kg) mothers and both were above the Scottish average for women of 64.8 kg (127). Again, the numbers analysed were small so this does not therefore exclude the possibility of a positive association between maternal obesity and OFC. Initially it does appear that within this sample there was a tendency to be overweight, however this reflects the fact that the recording of weight was at the time of birth of the index child.

### 6.8 Strengths of this study

One of the main strengths of this study has been its originality within the Scottish population, building on work carried out elsewhere worldwide. The processes undertaken and the problems encountered will allow us to develop strategies to analyse larger population groups. The unique availability of both the CLEFTSiS dataset and datasets covering maternal information, which could then be linked together allowed this study to be performed for the first time in Scotland and will act as a pilot for future research in this area.

As this study was population-based and utilised data covering a considerable period of time, it is therefore relatively robust against selection bias. The extremely positive presence of the HIC team and their ability to provide a substantial group of matched controls was a great advantage in this study.

### 6.9 Weaknesses

Although all babies born with OFC between 1990 – 2010 were included in this study, this still gave a relatively small sample size, particularly when considering that only around 5% of individuals in the Scottish population are diabetic. The results therefore may not be representative of the whole Scottish population.

The CLEFTSiS database is an accurate record of births with OFC, however there is a potential source of information bias where some cases of isolated CP may not have been diagnosed at birth and therefore not recorded in the dataset.

Another limitation of this study was that cleft types could not be analysed separately due to low numbers. Different types of OFC may have differing aetiological bases and this effect will be masked where the groups are analysed together.

The datasets were found to be incomplete in some areas, particularly regarding the potential confounding factors of smoking and alcohol intake. This means that their effect could not be separated. Maternal BMI could not be analysed due to poor reporting of BMI, height and weight.

## CHAPTER SEVEN: CONCLUSIONS AND RECOMMENDATIONS

The null hypothesis that the information held within the CLEFTSiS dataset and other maternal datasets within NHS Tayside has a high level of completeness which is required for accurate assessment and linkage must be rejected, as data sets were found to be poorly populated with data regarding several maternal characteristics, including smoking status and BMI.

The null hypothesis that maternal diabetes confers no increase in the risk of children being born with cleft lip and/or cleft palate in the population of Tayside, Scotland, must be accepted in this study.

The null hypothesis that maternal obesity confers no increase in the risk of children being born with cleft lip and/or cleft palate in the population of Tayside, Scotland, must also be accepted in this study.

Larger population-based database linkage studies would be indicated to ascertain whether a positive association between maternal diabetes and obesity and OFC exists in the Scottish population, as it is seen to in other population groups. The CLEFTSiS dataset is an invaluable source of information in the pursuit of this goal; and the CHI system in Scotland that allows record linkage to other relevant medical data has made this study possible.

This pilot project revealed a surprisingly low prevalence of reported diabetes among mothers of children with clefts of the lip and palate. As a result the numbers of mothers of CLP patients or CP patients that had a diagnosis of type 1, Type 2 or

gestational diabetes was extremely small and rendered the study under powered and therefore subject to statistical error.

Based on these results, a power calculation would enable a more reliable estimate of the size of the population and the number of clefts required to determine whether there is any relationship between maternal diabetes and the risk of having a child with a cleft lip and/ or palate.

However, as there were found to be large discrepancies in the completeness of the data sets analysed, there is a lack of robust data upon which to base a larger study at present. Prior to any such study being undertaken, datasets would require inspection to ensure that they are comprehensive enough to enable meaningful analysis. Including a larger volume of data by extending the timescale of data collection may be possible in the future.

Hospital-based databases must be fully complete where possible. This will rely on accurate and conscientious recording of maternal information at a clinical level, and therefore requires adequate provision of time and staff training.

Future studies should include more detailed information regarding:

- Confounding factors including smoking and alcohol intake
- Descriptive maternal information including BMI and type of diabetes
- The inclusion of all births with OFC – live, aborted and stillborn

This will allow a more accurate and detailed assessment of a possible association between maternal diabetes and obesity and OFC.

A worldwide effort to form a network of registries and research projects in both developed and developing countries is key in elucidating the importance of aetiological factors involved in the development of OFC. This will involve collaboration in order to achieve the large numbers required to tackle heterogeneity and find a true answer to our questions. The ultimate aim will be to obtain sufficient information on both genetic and environmental factors influencing OFC outcomes to enable strategies on primary prevention.

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# CHAPTER NINE: APPENDICES

## APPENDIX I

## CLEFTSiS NCAS Registration Form

**Registered with CLEFTSiS on …….**
**NCAS Information Leaflet Provided**
**Yes ☐ No ☐**

**PATIENT DEMOGRAPHICS**

Surname…… Address ……
Forename…… ……..
Gender M ☐ F ☐ City ……..
CHI Number …… Postcode ……..
Date of Birth…. ……. Registered GP……
Telephone Number…… Allergies/Alerts …….
Cleft Number ……. Health Board…….

Ethnicity

☐ Scottish
Scottish/British
☐ English
☐ Welsh
☐ Northern Irish
☐ British
Scottish/British
☐ Gypsy/Traveller
☐ Polish
☐ White Any other ethic group
☐ Any mixed or multiple ethnic group
☐ Pakistani, Pakistani Scottish/British
☐ Indian, Indian Scottish/British
☐ Bangladeshi, Bangladeshi
Scottish/British
☐ Chinese, Chinese Scottish/British
☐ Other Asian
☐ African, African Scottish/British
☐ Caribbean, Caribbean
Scottish/British
☐ Black, Black Scottish/British
☐ Other African
☐ Arab
☐ Any other Ethnic
☐ Refused/Not provided by patient
☐ Not known

**CLEFT DETAILS *(to be confirmed by Cleft Surgeon)***
(C=Complete, I= Incomplete)

| | **Patients Right** | | | | **Patients Left** |
|---|---|---|---|---|---|
| Lip | C / I / Nil | | | | C / I / Nil |
| Alveolus | | C / I / Nil | | C / I / Nil | |
| Hard Palate | | | C / I / Nil | | |
| Soft Palate | | | C / I / Nil | | |
| Simonarts Band (not on NCAS - for info only) | Yes/No | | | | Yes/No |

Submucous Cleft Yes ☐ No ☐
Non Cleft VPI Yes ☐ No ☐
Other ……

**LAHSHAL Code** ..... ***(Record within patient demographics notes field)***
**CLEFT Type:** BCL / UCL / CP / BCLP / UCLP

**COMORBIDITY DETAILS**

| | | | | | |
|---|---|---|---|---|---|
| Craniofacial Disorder | Yes ☐ | No ☐ | Other Medical | Yes ☐ | No ☐ |
| Di George Syndrome | Yes ☐ | No ☐ | Other Syndrome Recorded | Yes ☐ | No ☐ |
| Pierre Robin Syndrome | Yes ☐ | No ☐ | ***(Record further details under Encounters/Genetics)*** | | |

**REFERRAL DETAILS – FIRST CONTACT DETAILS**

Treatment Centre .....
Date of Referral .....
Date Referral Received .....
Referral Source ☐ Post natal
☐ Ante natal
☐ Late Referral

Clinician In Charge ***(Cleft Surgeon)*** .....

Reason for Referral .....
Referred by .....
Referred from .....
Referring Health Board .....

Referral Outcome ☐ Telephone call within 24 hours of referral
☐ Visit to patient within 24 hours of referral
Referral Outcome Date .....

**FAMILY HISTORY**

**History of Cleft**

| | | | |
|---|---|---|---|
| Mother | Yes ☐ | No ☐ | |
| Father | Yes ☐ | No ☐ | |
| Other | Yes ☐ | No ☐ | Specify Relationship ..... |
| Consanguineous | Yes ☐ | No ☐ | Specify Relationship ..... |
| Male Siblings | Yes ☐ | No ☐ | |
| Female Siblings Yes ☐ | No ☐ | | |
| Twin affected | Yes ☐ | No ☐ | |

**PARENTAL DETAILS/PREGNANCY DETAILS/BIRTH HISTORY**
*(not collected on NCAS – for information only)*

**Parental Details**
Age of Mother ..... Age of Father .....
Occupation of Mother ..... Occupation of Father .....

**Pregnancy Details**
Alcohol Units per week .....

| | |
|---|---|
| Drugs | Give Details …….. |
| Smoking | No/per week …….. |
| Illnesses | Give Details …….. |
| Trauma | Give Details …….. |
| Vitamins/Herbal remedies | Give Details …….. |
| | |
| **Birth History** | |
| Birth Number | Single/Multiple |
| Twin Type | Identical/Non-Identical |
| Twin Affected | Gender …….. |
| Gestation | Weeks …….. |
| Head circumference | ……. cm |
| Weight at birth | …… kg |
| Length at birth | ……. cm |

**APPENDIX II**

# CLEFTSiS

**(National Managed Clinical Network for Cleft Services in Scotland)**

The CLEFTSiS Register records details of all patients in Scotland with cleft lip and/or palate. The Register also contains the records which are required to monitor your child's treatment and compare their outcome with others to ensure they are receiving the best treatment possible. The register is maintained on behalf of CLEFTSiS by the Tayside University Hospitals NHS Trust.

The information is confidential, and will be stored securely. It will be linked through a unique CLEFTSiS number. Only professional members of the Network will have access to it if they have a particular need to use the information. Access will be through the CLEFTSiS Administrator.

The records taken include photographs, sound and video recordings of speech, dental impressions, and x-rays. The records are stored as part of the Register and will be anonymised when they are used to compare their outcome with others.

## *CONSENT FORM* for inclusion on the CLEFTSiS Register and for Records

Your child has been diagnosed as having a cleft lip/cleft palate/cleft lip and palate. Whatever type of cleft your child has it is important that he/she is seen regularly for check ups. These check-ups will be arranged for him/her to be seen by different specialists at their hospital clinics or at a combined clinic. With your permission, we would like to place details of your child on the CLEFTSiS register in order to:

a. make sure that s/he is given appointments for regular check ups.
b. share information about your child with other health professionals who are involved in their care.
c. to plan services and help our understanding of the condition through audit under the control of the executive support research by providing anonymous information.

Your are welcome to see what information is held on the register by contacting the CLEFTSiS Administrator at the address below, and you can ask for help in

understanding what it says. There may be a charge for this in accordance with the subject access arrangements for the Data Protection Act 1998. You are free to have your child's name and details removed from the register at any time, but it will mean we cannot use this information about your child to plan future services and to ensure that we do not lose contact with you.

***I do / do not consent to having my child's name on the CLEFTSiS register.***

***I do /do not consent to having CLEFTSiS records taken of my child.***

Child's Full Name:……
DOB:…….

Parent's Name:…….

Address:……..

……..

…….. Postcode: ……..

1st
signature:……Date:……

(At birth or at first combined clinic)

*The CLEFTSiS registry is maintained at Perth Royal Infirmary by:*

*The CLEFTSiS Network , Room 17, Admin Block, Perth Royal Infirmary PH1 1NX*

*Tel: 01738 473508 Fax: 01738 473278*